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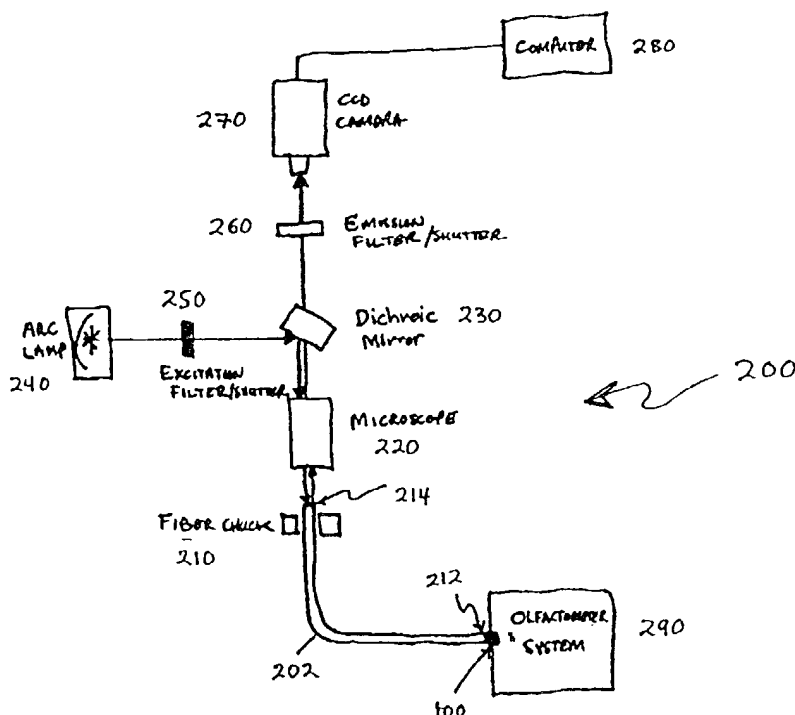
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ^{7} :		(11) International Publication Number:	**WO 00/13004**
G01N 21/64	**A2**	(43) International Publication Date:	9 March 2000 (09.03.00)

(21) International Application Number: PCT/US99/19624

(22) International Filing Date: 26 August 1999 (26.08.99)

(30) Priority Data:
09/140,352   26 August 1998 (26.08.98)   US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMBINATORIAL POLYMER SYNTHESIS OF SENSORS FOR POLYMER-BASED SENSOR ARRAYS

(57) Abstract

A combinatorial synthesis method for fabricating unique families of discrete copolymer sensors and copolymer gradient sensors is provided. The method employs combinatorial copolymer synthesis of discrete monomer or oligomer combinations as well as spatially-varying combinations for generating large numbers of analyte-discriminating sensors from a limited selection of initial monomer and oligomer compositions. The method can be applied to either analyte-specific sensors or sensor arrays or semi-selective sensors and cross-reactive sensor arrays which employ virtually any known physic-ochemical transduction mechanism for detecting analytes. Since the analyte response characteristics of such copolymer sensors are not limited to a linear proportional ratio of the monomer or oligomer combinations employed, the resulting copolymer sensors provide for increased diversity in sensor and sensor array response characteristics for discriminating between a variety of materials and for detecting and identifying analytes in fluid samples.

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COMBINATORIAL POLYMER SYNTHESIS OF SENSORS
FOR POLYMER-BASED SENSOR ARRAYS

FIELD OF THE INVENTION

This invention relates to a method for combinatorial polymer synthesis of sensors for polymer-based
5   sensor arrays and, more particularly, to copolymer sensor compositions, their synthesis and application
in chemical sensor arrays.

BACKGROUND OF THE INVENTION

Combinatorial synthesis methods are known in the art and have been successfully employed in a
growing number of research areas including drug discovery [E.M. Gordon, et al., *Acc. Chem. Res.*
10   29:144 (1996); M.J. Plunkett, et al., *Scientific American*, April 1997, p. 69], drug optimization [Hobbs, et
al., *Acc. Chem. Res.* 29:114 (1996)], complex sequence-selective receptor molecule synthesis [W.C.
Still, *Acc. Chem. Res.* 29:155 (1996)], catalytic antibody production [P.G. Schultz, et al., *Science*
269:1835 (1995)], inorganic superconductor synthesis [X.D. Xiang, et al., *Science* 268:1738 (1995)],
inorganic magnetoresistance materials synthesis [G. Briceno, et al., *Science* 270:273 (1995)] and
15   inorganic luminescent materials synthesis [E. Danielson, et al., *Nature* 389:944 (1997)].

Photochemical approaches to combinatorial synthesis have been disclosed in a number of references
[see U.S. Patent Nos. 5,288,514 and 5,545,568 to Ellman; S.P.A. Fodor, et al., *Science* 251:767 (1991);
U.S. Patent No. 5,424,186 and PCT Publication No. WO 92/10092 to Fodor, et al; U.S. Patent No.
5,527,681 to Holmes, et al; J.W. Jacobs, et al., *TIB TECH* 12:19 (1994); U.S. Patent No. 5,412,087 to
20   McGall, et al; and U.S. Patent No. 5,143,854 to Pirrung, et al;]. In these methods, photochemical
syntheses of large arrays of biologically active compounds which are immobilized on solid substrates
are disclosed using conventional photolithography methods. With these methods, immobilized polymer
compounds are assembled in step-wise fashion using spatially localized photolabile masking groups
in combination with conventional masks for selective illumination, blocking, cleavage, coupling and
25   photochemical reaction of functional groups with immobilized polymer backbones. These references
disclose methods for fabricating arrays of diverse biological compounds for chemical assays by way of

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a sequential build-up of molecular fragments onto a polymer backbone in a series of masking steps and photoinitiated reactions.

Lundstrom, et al., [l.Lundstrom, et al., *Nature* 352:47 (4 July 1991); I. Lundstrom, *Sensors and Actuators*, A56:75 (1996)] have disclosed a quasi-combinatorial method for fabricating chemical sensors
5   in which simple sets of binary masks are used to produce a patterned, field-effect transistor (FET) array surface by sequential masking and evaporative deposition of layers of different metals on a semiconductor substrate. The metals are subsequently heated to combine the vapor deposited materials by either reaction or alloying to form a matrix of different compounds. This method provides for production of thin-film chemical-FET (ChemFET) sensor arrays with a variety of different film
10   compositions deposited in distinct areas of the array.

Xiang, et al., [X.D. Xiang, et al., *Science* 268:1738 (1995)] have disclosed a quasicombinatorial method for fabricating superconducting thin films using a similar approach to that of Lundstrom, et al. The disclosed method relies on a combination of conventional thin film deposition methods and physical masking by reacting layers of deposited materials to generate a spatially defined library of solid-state
15   thin film compositions of electronic, magnetic, or optical materials by parallel synthesis. Danielson, et al., [E. Danielson, et al., *Nature* 389:944 (1997)] have disclosed a quasi-combinatorial approach similar to that of Lundstrom and Xiang for rapidly synthesizing and prescreening candidate luminescent materials whose properties cannot be predicted by theoretical models. This method also relies on conventional physical masking and deposition of multiple thin film layers for production of a variety of
20   layered combinations for subsequent reaction and synthesis via thermal oxidative annealing of the as-deposited, multi-layered, solid-state structures to form libraries of candidate compounds for evaluation.

Schultz [PCT Application No. PCT/US95/13278 (Ins. Publication No. WO 06/11878)] discloses a method for parallel deposition, synthesis and screening of arrays of diverse compounds at predetermined
25   locations on a solid substrate. The method relies on delivering a plurality of components in any stoichiometry, or stoichiometric gradients, to predefined regions on a substrate and simultaneously reacting the components to form a plurality of different compounds. Disclosed methods for delivery of components include thin-film deposition techniques in combination with masking and photolithography, and dispensing methods where droplets or powders are delivered by a suitable dispenser. Disclosed
30   methods for reacting the components include solution-based synthesis, template directed synthesis, photoinitiated reactions, polymerization, heating, annealing and crystallization. The disclosed method is used for rapid parallel synthesis and screening to dentify optimized compositions through measurement and comparison of their known targeted properties.

With the above disclosed methods, various permutations and combinations of candidate materials,
35   compounds, chemical functionality or elements are experimentally synthesized and screened to identify

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optimum compositions or properties for a known application. With these methods, combinatorial synthesis techniques are typically used to efficiently synthesize, test and identify materials in prescreening a large number of organic or inorganic compounds for an optimum targeted reactivity or performance characteristic where the performance of candidate materials cannot be predicted by

5   theory. With these methods, compositions which do not demonstrate optimum performance or preferred properties are typically eliminated and discarded as undesirable or impractical candidate materials for the targeted application.

In the field of chemical sensor technology, a variety of chemical sensor formats and transduction mechanisms have been developed for detection of analytes in gaseous and liquid samples. These

10   sensors employ various sensing mechanisms, utilizing changes in chemical, physical, electrical, mechanical, optical, or thermal properties of sensor materials for detection and recognition of target analytes in contact [see *Chemical Sensors and Microinstrumentation*, ed. R.W.Murray, et al., American Chemical Soc. (Washington, D.C. 1989); R.W. Cattrall, *Chemical Sensors*, Oxford Univ. Press, (New York 1997); *Handbook of Chemical and Biological Sensors*, ed. R.F. Taylor, et al., Inst. of Physics

15   Publishing (Philadelphia 1996); G. Boisde, et al., *Chemical and Biochemical Sensing with Optical Fibers and Waveguides*, Artech House (Boston 1996); and *Surface-Launched Acoustic Wave Sensors*, M.Thompson, et al., J.Wiley & Sons (New York 1997).

A number of electrical transduction mechanisms have been employed for chemical sensing of target analytes. Electrical sensors have been employed where the sensor transduction mechanism is due to

20   changes in conductance, resistance, or interface potential. Conductivity sensors rely on adsorption of an analyte onto a sensor substrate interposed between two electrodes biased by an applied potential. With these sensors, the adsorbed analyte produces a change in charge carriers which creates a detectable change in current. Amperometric chemical sensors have been developed for measuring microamp currents produced by analyte adsorption between two sensor electrodes maintained at a fixed

25   relative potential.   In addition, amperometric sensors have been developed which rely on electrochemical reaction currents generated by redox reactions occurring at the sensor surface. Potentiometric sensors rely on interfacial electrochemical potentials created by binding or transport of charged analytes across thin membrane films which are designed with chemical binding or transport specificity to given analytes. In addition, potentiometric chemical sensors have been developed as

30   ion-selective electrodes which utilize half-cell reaction voltages for electrochemical detection of specific analytes.

U.S. Patent No. 4,717,673 to Wrighton, et al., discloses electrode applications of conducting polymers where reversible interaction of a polymer with an analyte produces a characteristic voltage. U.S. Patent No. 5,696,314 to McCaffrey, et al., discloses an amperometric electrode where a characteristic current

35   is produced by interaction of an analyte with an immobilized enzyme layer.

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Additional electrical-based transduction mechanisms have been employed in sensors which rely on changes in resistance, inductance or capacitance due to analyte sorption. U.S. Patent Nos. 5,607,573 and 5,417,100 to Miller, et al, disclose applications of conductive and dielectric polymers in electrodes where analyte sorption produces detectable changes in conductivity of the polymers. U.S. Patent Nos.

5   5,571,401 and 5,698,089 to Lewis, et al., disclose sensors and sensor arrays that employ chemically sensitive resistors comprised of non-conducting polymers and conductive materials which produce a detectable change in resistance upon exposure to analytes. H.V.Shurmer, et al., *Sensors and Actuators B* 4:29 (1991) and P.N. Bartlett, et al., *Sensors and Actuators A* 23:911(1990) and *Sensors and Actuators A* 20:287(1989) disclose gas sensors which employ conducting polymers for detection of

10   analyte vapors. U.S. Patent No. 5,417,100 to Miller, et al., discloses a conductive electrode vapor sensor comprised of a composite coating of a conductive polymer and a dielectric polymer which has an affinity for analyte vapors. U.S. Patent No. 5,312,762 to Guiseppi-Elie discloses a resistivity sensor which relies on a change in resistance of electroactive polymers when exposed to analytes.

More recently, electronic chemical sensors have been developed which rely on changes in solid-state

15   electronic properties due to adsorption of analytes on polymer-coated field effect transistor (FET) or metal oxide field effect transistor (MOSFET) surfaces [see Y. Ito, et al., *Sensors and Actuators B* 1-3:348 (1983); Miyahara, et al., *Sensors and Actuators B* 7:1 (1985); Hanazato, et al., *IEEE Trans.*, vol. ED-33(1):47 (Jan. 1986); Caras, et al., *Anal. Chem.* 57(9):1920-1925 (1985); I. Lundstrom, et al., *Nature* 352:47 (4 July 1991); H.M. McConnell, et al., *Science* 257: 1906 (1992); I. Lundstrom, *Sensors and*

20   *Actuators A* 56:75 (1996); U.S. Patent No. 5,466,348 to Holm-Kennedy]. U.S. Patent No. 4.909.921 to Ito discloses an electrochemical FET sensor for detecting chemical substances which employs a continuous hydrous polymer coating on an insulating layer and an enzyme-immobilized coating formed on a channel portion of the FET.

Mass-sensitive mechanical properties have also been employed as transduction mechanisms for

25   detecting target analytes. Piezoelectric sensors, such as quartz crystal microbalances or surface acoustic wave devices, have been employed which rely on an oscillating crystal that produces a shift in oscillating frequency due to sorption of analytes on a sensor surface [see W.P. Carey, et al., *Anal. Chem.* 58:3077 (1986); K. Yokoyama, et al., *Anal.Chem.* 65:673 (1993); M.Rapp, et al., *Fresenius J.Anal. Chem.* 352:699 (1995)]. In typical applications of such piezoelectric sensors, carefully selected

30   polymer coatings are applied to the surface for optimizing sensitivity and selectivity towards specific target analytes [see W.P. Carey, et al., *Anal. Chem.* 58:149 (1986); E.T. Zellers, et al., *Anal.Chem.* 67:1092 (1995); J.W. Grate; et al., *Anal.Chem.* 67:2162 (1995)]. Generally, such devices rely on selective interaction of an analyte with a thin film coating placed on an active surface of the sensor. By binding or partitioning of an analyte due to the presence of the surface coating, the resonant frequency

35   of the sensor is reduced in proportion to the increased mass of analyte. By proper selection of sorptive coatings, these sensors are capable of detecting analytes by measurement of changes in crystal oscillating frequency.

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U.S. Patent No. 4,596,697 to Ballato discloses a piezoelectric sensor array which employs a plurality of coated resonators embedded in a single piezoelectric crystal where reactive coatings are utilized for detection of target analytes. In U.S. Patent Nos. 5,719,324 to Thundat, et al., and 5,445,008 to Wachter, et al., micro-cantilever piezoelectric sensors are disclosed which rely on resonant frequency changes

5   in a micro-cantilever oscillated by a piezoelectric transducer where such changes are produced by adsorption of analytes on a microcantilever coated with a sorptive material.

Thompson, et al., have recently reviewed the state of the art of surface acoustic wave sensor ("SAW") technology [see M. Thompson, *Surface-Launched Acoustic Wave Sensors*, J.Wiley & Son (New York 1997)]. These devices are much more sensitive to oscillation frequency changes than conventional

10   quartz crystal microbalances due to the substantial increase in detectable frequency range available with SAW devices. In U.S. Patent Nos. 4,895,017 to Pyke, et al., 5,151,110 to Bein, et al., 5,235,235 to Martin, et al., and 5,25,704 to Mariani, et al., surface acoustic wave sensors are disclosed which rely on the change in resonant frequency of propagated surface waves due to adsorption of analytes by a sorptive surface coating. Surface acoustic wave sensor arrays which have employed a variety of

15   chemically selective coatings have been disclosed in U.S. Patent No. 5,464,608 to Lokshin, et al., and by Rapp, et al., [M.Rapp, et all, *Fresenius J.Anal.Chem.* 352:699 (19995)]. Criteria for optimal selection of SAW polymer coatings have been discussed by W.P. Carey, et al., *Anal. Chem.* 58: 149 (1986), E.T. Zellers, et al, *Anal.Chem.* 67:1092 (1995), and J.W. Grate, et al., *Anal.Chem.* 67:2162 (1995).

Optical and electro-optical mechanisms have also been employed as transduction methods for sensors

20   that rely on changes in optical response for detecting analytes [see G.Boisde and A.Hammer, *Chemical and Biochemical Sensing with Optical Fibers and Waveguides*, Artech House (Boston 1996)]. Thus, optical absorbance, emittance, transmittance, reflectance, luminescence, interference, polarization, or surface plasmon resonance may be monitored for detecting analytes. With these methods, the sample or sensor is typically illuminated with incident light energy having a certain optical or spectral

25   characteristic and the sensor-analyte interaction produces an optically detectable response which is indicative of the analyte.

Optical sensors and sensing methods have been disclosed which rely on changes in refractive index due to sorption of analytes at a sensor surface Esee Kawahara. et al., *Anal. Chim. Acta* 151:315 (1983); Sutherland, et al., *Anal. Lett.* 17:43 (1984); Guiliani, et al., *Sens. Actuat.* 6:107 (1984); V.S.Y. Lin, et al.,

30   *Science* 278:840(31 Oct. 1997)]. Optical sensors which employ interferometric measurements have been disclosed by Butler [*Appl.Phys.Lett.* 45:1107 (1984)] and Dessy [*Anal.Chem.* 57:1188A (1985)]. U.S. Patent No. 5,606,633 to Groger, et al., discloses a transduction method which relies on monitoring the intensity ratios of TM and TE polarization of an incident light beam where attenuation of TM polarization occurs due to adsorption of an analyte at the sensor surface.

Fiber optic sensors which employ optical fibers, fiber optic arrays and light absorbing dyes are particularly useful as optical sensors and have been disclosed by a number of workers [see W.R. Seitz. C.R.C. Crit. Rev. Anal. Chem. 19:135 (1988); *Molecular Luminescence Spectroscopy, Methods and Applications*, ed. S.G. Schulman, J. Wiley & Sons (New York 1988); D.R. Walt, et al., in *Chemical*

5   *Sensors and Microinstrumentation*, ACS Symposium 403, p. 252, American Chemical Soc. (Washington, D.C. 1989); S.M. Barnard, et al., *Environ. Sci. Technol.* 25(7):1301 (1991); S.M. Barnard, et al., *Nature* 353:338 (26 Sept 1991); O.S. Wolfbeis in *Fiber Optic Chemical Sensors*, vol. 2, CRC Press (Boca Raton, FL 1991)]. U.S. Patent Nos. 5,244,636 and 5,250,264 to Walt, et al., disclose methods for attaching a plurality of polymer-dye combinations, representing multiple chemical functionalities, as

10   optical sensor elements in a fiber optic array where sensor elements are selected due to their sensitivity and selectivity for particular analytes. In order to provide for discrimination and detection of analytes of interest by these methods, sensor elements typically must be capable of producing a characteristic optical response in the presence of an analyte when subjected to excitation light energy.

Surface plasmon resonance methods have recently been disclosed as useful optical transduction

15   methods for chemical sensors in detecting of analytes [see K. Matsubara, et al., *Applied Optics* 27:1160 (1988); D.C. Cullen, et al., *Sensors and Actuators* BI:576 (1990); Villuendas, et al., *Sensors and Actuators* A21 :1142 (1990); I.Garces, et al., *Sensors and Actuators* B7:771 (1992); J.Melendez, et al., *Sensors and Actuators* B39(1-3):375 (1997)]. U.S. Patent Nos. 5,255,075 to Cush, and 5,359,681 or 5,647,030 to Jorgenson. et al]. Cush discloses a transduction method which relies on an angular shift

20   in the resonance angle of incident polarized light as a means for sensing analyte absorption on the sensor. U.S. Patent Nos. 5,359,681 and 5,647.030 to Jorgenson, et al., disclose use of thin film coatings as either dynamic range controlling layers, for modifying the range in indices of refraction of sensors, or reactive layers, for interacting with specific analytes to produce a detectable shift in an effective surface refractive index due to absorption of an analyte by the film layer.

25   While the above sensing methods typically rely on the sensitivity of a particular sensor to a specific analyte, more recently, chemical sensor array formats have been developed, in which a series of discrete, cross-reactive sensing regions are used in conjunction with pattern recognition schemes for detecting and identifying a broad range of analytes. With this cross-reactive sensor array approach, there is no need to employ sensor elements which are specifically sensitive to a particular analyte as

30   the combined sensor responses within the sensor array are used for producing a characteristic array response to a diverse selection of analytes.

A variety of sensor transduction methods have been employed in such cross-reactive sensor arrays where sensor array elements may utilize any determinable physicochemical phenomenon which is characteristic of the sensor-analyte interaction and which provides a discriminating and detectable

35   response. Thus, changes in electrical properties, such as conductance, resistance, interface potential, electrochemical half-cell voltage or reaction currents, optical properties, such as absorption, emission,

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reflectance, transmittance, polarization, interference, or surface plasmon resonance, physical changes, such as swelling or contraction, mass changes due to surface sorption of analytes, or any other measurable physicochemical analyte-sensor interaction phenomena may be utilized for sensing analytes.

5   Such cross-sensing sensor array methods have been applied to surface acoustic wave sensor formats [J. Grate, et al., *Anal. Chem.* 63: 1719 (1991); S. Rose-Pehrsson, *Anal. Chem.* 60:2801 (1988); E. Zellers, et al., *Anal.Chem.* 67:1092 (1995)], electrochemical sensor formats [J. Stetter, et al., *Anal. Chem.* 58:860 (1986)], conductive polymer sensor formats [J. Hatfield. et al., *Sens. Actuators* B 18-19:221 (1994); M. Freund, et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:2652 (1995); Lewis, et al., U.S.

10   Patent Nos. 5,571,401 and 5,698,089], and piezoelectric sensor formats [W. Carey, et al., *Anal. Chem.* 60:2801 (1988); T. Thundat, et al., *Anal.Chem.* 67:519 (1995)].

More recently, Walt, et al., have disclosed cross-reactive fiber optic array sensors which employ diverse polymer-dye combinations in conjunction with fiber optic bundles or arrays for detecting a variety of analytes [see J. White, et al., *Anal. Chem.* 68:2191 (1996) and T.A. Dickinson, et al., *Nature* 382:697

15   (1996)]. U.S. Patent No. 5,512,490 to Walt, et al., discloses a cross-reactive, fiber optic sensor array comprised of a plurality of polymer-dye combinations as sensor elements where the sensors are semi-selective for particular analytes. The disclosed array and detection method provides for detecting a variety of materials by applying pattern recognition techniques to the composite sensor array response to analytes.

20   Polymeric materials have particular utility as either sensor element matrices or surface coatings applied to sensor elements for enhancing sensor discriminating capabilities towards analytes. Thin polymer films are effective in modifying and enhancing sensor reactivity, response times, sensitivity, specificity and selectivity and may be successfully employed in most every sensor format. Due to the substantial flexibility and control of polymer structure and properties, polymer films may be chemically designed to

25   preferentially adsorb or repel particular analytes, thereby significantly improving selectivity of a sensor. Polymer thin films may enhance chemical selectivity by either altering the partitioning of analytes or interferants between the sample medium and sensor or modifying the transport properties of analytes to the sensor surface.

A variety of polymeric materials have been employed in cross-reactive sensor arrays as semi-selective

30   sensor elements to provide for discrimination between target analytes. In these applications, analyte discrimination may be achieved by exploiting various polymer properties such as analyte solvation and partition coefficients [J.W. Grate, et al., *Anal. Chem.* 67:2162 (1995); J.W. Grate, et al., *Sens. Actuators* B3:85 (1991)] molecular polarity [J. White, et al., *Anal. Chem.* 68:2191 (1996); T.A. Dickinson, et al., *Nature* 382:697 (1996)], dimensional changes or swelling upon exposure to analytes [J. White, et al.,

35   *Anal. Chem.* 68:2191 (1996); T.A. Dickinson, et al., *Nature* 382:697 (1996); M.C. Lonergan, et al., *Chem.*

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Mater. 8:2298 (1996); S.M. Barnard, et al., *Environ. Sci. Technol.* 25:1301 (1991)], changes in conductivity upon exposure to an analyte [W.P. Carey, et al., *Anal. Chem.* 58:3077 (1986); S. Unde. et al., *Adv. Mater. Opt. Elect.* 6:151 (1996)], and analyte sorption characteristics [R.A. McGill, et al., *CHEMTECH* 9:27 (1994); J.W. Grate, et al., *Anal. Chem.* 68:913(1996)].

5   Irrespective of the transduction means employed for chemical sensors, a potentially significant limitation of current chemical sensors and sensor technology is the limited selection and choice of polymeric sensor materials and sensor coatings which can provide discriminating responses for detecting analytes of interest by producing a unique, determinable, characteristic sensor response to a variety of analytes. Thus, a new family of sensor materials which provides for expanding the number and diversity of

10   available sensor elements for use as discrete chemical sensors or in chemical sensor arrays is required for increasing the capabilities of chemical sensor arrays and improving the selectivity, sensitivity and detection of a variety of target analytes with such arrays.

SUMMARY OF THE INVENTION

In general, the present invention provides for the development of families of unique chemical sensing

15   elements for use either as discrete sensors or as semi-selective sensor elements in cross-reactive chemical sensor arrays and a method for fabricating the same. The innovative method relies on combinatorial polymer synthesis of copolymer sensors from two or more distinct monomers, oligomers, or their derivatives, where the monomers or oligomers are polymerized in either discrete composition ratios or from continuously varying composition ratios. The synthesis of diverse polymers may likewise

20   be performed on beads as starting meterials, leading to combinatorially functionalized beads. The method provides for the rapid development and generation of large families of innovative, semi-selective polymer sensor elements for use in chemical sensor arrays from a limited number of available monomer or oligomer starting materials. By increasing the number of unique sensor element types in a sensor array, an increasing amount of discriminating characteristic sensor response information is provided for

25   collection and analysis in detecting analytes of interest. By increasing the amount of discriminating information which is generated by such sensor arrays for processing by neural networks or other computer-based pattern recognition schemes, the detection capability and sensitivity of the sensor array to a variety of target analytes is greatly enhanced.

The combinatorial polymer synthesis method for fabricating chemical sensors of the present invention

30   further provides for synthesizing a large family of copolymer sensors that produce more distinctive and characteristic responses to the presence of analytes of interest than would be anticipated when two or more different monomers or oligomers are proportionally combined in varying composition ratios and copolymerized to produce a new copolymer sensor matrix material. The response characteristics of such combinatorial polymer sensors are thus more unique and distinctive than the mere proportional

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combination of starting monomer or oligomer materials would suggest. The ability to create such variations in the responses of sensor elements to target analytes, from copolymerization of two or more starting monomers or oligomers in varying proportions, represents a potentially powerful and useful approach for chemical sensor array development and fabrication for chemical sensing of an increasingly

5   large number of target analytes.

The polymer sensor of the present invention can take on a variety of configurations for generating chemical sensor diversity through the combinatorial polymer synthesis method. In one embodiment, predetermined composition ratios of two or more distinct monomers or oligomers are combined and polymerized to form a unique copolymer matrix material useful as a polymer sensor element. In this

10   embodiment, specific monomer or oligomer composition ratios produce discrete copolymer sensor elements which exhibit unique and characteristic responses for given target analytes. As more fully outlined below, this can be done in a variety of ways, including polymerizing the mixtures onto discrete sites of a substrate, or onto microspheres distributed onto the substrate, or by polymerizing the mixtures into beads which are distributed on the substrate. In an alternative embodiment, prepolymer mixtures

15   of continuously varying ratios of two or more monomers or oligomers may be copolymerized to form a continuous copolymer gradient sensor which has a spatially varying polymer structure. In this embodiment, the continuous variation in composition ratio during polymerization of two or more distinct monomers or oligomers to form a copolymer sensor matrix creates a gradient sensor having a spatially varying copolymer structure with unique analyte response characteristics which vary according to spatial

20   location along the copolymer structure gradient. With such gradient sensors, each location within the copolymer gradient provides a uniquely characteristic response to target analytes. With these embodiments, by utilizing predetermined varying composition ratios of a relatively small group of monomers or oligomers and combining them in predetermined permutations and combinations of prepolymer mixtures for copolymerization in differing proportions, an expansive set of unique

25   combinatorial polymer sensors are made available in which each sensor exhibits a unique characteristic response to a variety of analytes.

The combinatorial polymer sensors and sensor arrays of the present invention may be employed with a variety of detection or transduction mechanisms for providing a characteristic and discriminating response to target analytes of interest. Sensors of the present invention have utility in a variety of

30   sensing applications as either discrete sensors or in sensor arrays where, upon exposure of the copolymer sensors to analytes, interaction of analytes with the sensors produces a unique, characteristic, and determinable, time-varying, physicochemical response, said response detected by measuring a property selected from a group consisting of mass, temperature, heat, voltage, current, polarity, intensity, refractive index, polarization, phase, wavelength, frequency, periodicity, and dimension,

35   said response being indicative of the presence or absence of certain analytes.

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By way of example, optical sensors and sensor arrays of the present invention may employ characteristic spectral response signatures, such as variation in absorption, emission, transmission or reflection intensities or wavelengths, or, alternatively, spectral variations in interference, polarization, refractive index, periodicity, phase or frequency for identification and detection of analytes. Alternatively,
5   combinatorial polymer sensor coatings may be employed with piezoelectric sensors, such as quartz crystal microbalances, surface acoustic wave (SAW), or bulk acoustic wave (BAW) sensors and sensor arrays, where such coatings provide for discrimination and detection of analytes due to mass changes from sorption of analytes. In addition, analytes may be identified and distinguished by employing combinatorial copolymer sensors and sensor arrays fabricated from dielectric and conducting polymers
10 where sorption of target analytes produce unique and characteristic changes in resistance, conductance, capacitance, voltage or current. Other embodiments include combinatorial polymer sensors which rely on discriminating between temperature changes or heat generated due to adsorption of target analytes with micro-calorimeter sensors, ion-selective electrochemical sensors which rely on electrochemical reaction half-cell voltages or reaction currents, or measuring dimensional, mechanical or other
15 physicochemical changes in a sensor due to analyte sorption.

In one embodiment, where characteristic optical response signatures are employed as an analyte discriminating means, combinatorial polymer sensors may be coupled with fiber optic arrays, excitation light sources and optical detectors. In this embodiment, the sensors and sensor arrays of the present invention are typically fabricated by immobilizing a copolymer sensor at the end of an optical fiber or a
20 fiber optic array, comprised of either a fiber optic bundle, a preformed, unitary fiber optic array or imaging fiber comprising a plurality of individual fibers. In one preferred embodiment, a dye compound may be incorporated within a copolymer matrix which is photodeposited by photoinitiated copolymerization of two or more different monomers or oligomers. While solvatochromic dyes have particular utility in these sensor and sensor array embodiments, any indicator dye may be employed with such sensors to
25 produce a characteristic optical response upon exposure to excitation light in the presence of analytes.

Where dye compounds are employed in copolymer optical sensors and sensor arrays, solvatochromic dyes are particularly useful since these dyes are known to exhibit shifts in emission wavelength depending on the polarity of the local polymer matrix-analyte environment. When solvatochromic dyes are employed, an analyte contacting the copolymer sensor element typically alters the polymer
30 microenvironment, generally producing a change in polarity and giving rise to a complex temporal change in the fluorescence signal of the sensor when subject to excitation light energy. The phase, intensity, and shape of these temporal outputs depend directly on the physical and chemical nature of the particular polymer matrix in which the dye is entrapped.

While the copolymer sensors of the present invention may be employed as either analyte-specific
35 sensors or as analyte-specific sensor elements in a sensor array where specific sensor elements are utilized for detecting specific analytes, the sensors and sensor arrays of the present invention would

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have particular utility as semi-selective sensor elements in cross-reactive sensor arrays. Semi-selective sensors are sensors which interact with a large number of analytes and do not interact exclusively with a specific analyte. Such sensors provide for a broad range of responses to a variety of analytes. That is, analytes can be detected and/or identified by a "signature" of individual responses on the sensors,

5   similar to the manner in which "electronic noses" work. Cross-reactive sensor arrays are arrays which comprise a number of primary sensor elements which may either be selective to specific analytes or semi-selective to a variety of analytes. Typically, such arrays comprise a number of individual, broadly responsive sensor elements which are semiselective. The sensing elements of such arrays can provide a spatially distributed, time-varying response to a variety of analytes and are differentially responsive to

10 a large number of analytes. The application of semi-selective sensor elements in cross-reactive sensor arrays thus enables using sensors and sensor materials which may not otherwise be useful as analyte specific sensors in conventional sensor array applications. The combinatorial polymer synthesis method for fabricating copolymer sensors of the present invention is particularly suitable for creating semi-selective sensors for these sensor applications. By providing new classes and families of

15 copolymer sensors using the combinatorial polymer synthesis method of the present invention, expanded numbers of unique and diverse chemical sensors are thus provided for in a variety of sensor transduction mechanisms so as to increase the discriminating capabilities of chemical sensors and sensor arrays by increasing the diversity in the characteristic temporal responses of such sensors to a variety of analytes.

20 **BRIEF DESCRIPTION OF THE DRAWINGS**

This invention is pointed out with particularity in the appended claims. Other features and benefits of the invention can be more clearly understood with reference to the specification and the accompanying drawings in which:

Fig. 1 is a schematic diagram of the photodeposition system used for fabricating the copolymer sensors
25 of Examples 1-3;

Fig. 2 is a schematic block diagram of the apparatus and instrumentation used for measuring the optical response characteristics of copolymer sensors of the present invention;

Fig. 3 is a fluorescence image of a sensor array comprising eight discrete PS802/MMA copolymer sensors of the present invention;

30 Figs. 4a-d compare the temporal fluorescence responses of eight discrete PS802/MMA copolymer sensors with various MMA additions upon exposure to a) benzene, b) hexane, c) 2-propanol, and d) ethyl acetate;

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Figs. 5a-b compare a typical fluorescence im'age of a PS802/PS901.5 gradient copolymer sensor of the present invention in Fig. 6a with a PS901.5 non-combinatorial polymer sensor in Fig. 6b;

Figs. 6a-b compare temporal fluorescence· responses observed at consecutive points along a PS802/PS901.5 gradient copolymer sensor in Fig. 6a with the responses of a PS901.5
5   non-combinatorial polymer sensor in Fig. 6b upon exposure to benzene;

Fig. 7a-b compare temporal fluorescence responses observed at consecutive points along a PS802/PS901.5 gradient copolymer sensor in Fig. 7a with the responses of a PS901.5 non-combinatorial polymer sensor in Fig. 7b upon exposure to methanol;

Figs. 8a-b compare a fluorescence image of a PS802/MMA gradient copolymer sensor of the present
10 invention in Fig. 8a with a PS802 non-combinatorial polymer sensor in Fig. 8b;

Figs. 9a-b compare temporal fluorescence responses observed at consecutive points along a PS802/MMA gradient copolymer sensor in Fig 9a with the response of a PS802 noncombinatorial polymer sensor in Fig. 9b upon exposure to hexane;

Figs. 10a-b compare temporal fluorescence responses observed at consecutive points along a
15 PS802/MMA gradient copolymer sensor in Fig. 10a with the response of a PS802 non-combinatorial polymer sensor in Fig. 10b upon exposure to methanol;

Figs. 11a-b compare temporal fluorescence responses observed at consecutive points along a PS802/MMA gradient copolymer sensor in Fig 1 la with the response of a PS802 non-combinatorial polymer sensor in Fig. 1 lb upon exposure to benzene;

20 Fig. 12 is a dendogram plot comparing the dissimilarity in response of discrete copolymer sensors to benzene vapor; and

Fig. 13 is a multidimensional scaling plot comparing the dissimilarity in responses of a gradient copolymer sensors and control sensor.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

25 1.   Combinatorial Synthesis of Copolymer Sensors
The copolymer sensor elements and sensor arrays of the present invention are fabricated by thermal polymerization, photopolymerization, crystallization or precipitation of from a precursor solution, comprising of a mixture of monomers or oligomers in varying compositional ratios, to form copolymers having distinguishing polymer structures. Particularly useful sensor candidates for use in the copolymer

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sensors and sensor arrays of the present invention are monomers, oligomers or prepolymers which, when polymerized, exhibit either characteristic swelling responses, characteristic polarity differences, characteristic electrochemical potentials, characteristic electrochemical currents, characteristic conductance, characteristic solvation effects, characteristic partition coefficients, characteristic sorption

5   properties or characterishc structural features upon exposure to various analytes due to combinations of such materials in unique composition ratios. In selecting candidate monomers, oligomers, and copolymers as sensor materials and evaluating candidates based on desirable swelling, polarity, solvation, partition and adsorption characteristics, two particularly useful references are: R.A. McGill, et al., *Chemtech*, September 24, 1996, p 27-37 and J.W. Grate, et al., *Anal. Chem.* 68:913-7 (1996),

10 hereby incorporated by reference.

The choice of copolymer sensor families used to form the sensor array elements in a particular sensor array is primarily determined based on the transduction mechanisms to be employed, the analytical purposes of the sensor, and the analytes which are anticipated targets for detection. Features such as polymer sensor matrix polarity, chemical structure, chemical functionality, surface area, pore size,

15 swelling characteristics, partition coefficients, solvation properties, or chemical sorption behavior, either separately or in combination, contribute to the characteristic response signature of a given polymer sensor type. In one embodiment, sensor materials which are permeable or semi-permeable to vapor or liquid analytes are preferred. In another embodiment, sensor materials that swell upon contact with vapor or liquid analytes are preferred. In general, monomer, oligomer and polymer materials which

20 have unique polarity; structure, pore size, surface area, functionality, analyte partitioning features or adsorption characteristics are particularly useful for copolymer sensor matrices of the present invention.

The combinatorial polymer method for fabricating sensors of the present invention can utilize polymerizable prepolymer mixtures of any monomers or oligomers that copolymerize by either photopolymerization or thermal polymerization and which are either themselves miscible or are miscible

25 in a solvent. The method provides for a wide diversity of compositions selected from diverse polymer families such as vinyl or olefin polymers, including free radical polymers or addition polymers, and condensation polymers, where any combination or permutation of two or more distinct monomers or oligomers may be polymerized to form linear, branched or crosslinked copolymers. By utilizing either discrete compositional ratios or a varying compositional ratio gradient of two or more monomers or

30 oligomers in the prepolymer mixture, copolymerized sensor matrices may be fabricated having unique, distinguishable, and characteristic swelling behavior, polarity, conductivity, resistivity, piezoelectric properties or chemical adsorption characteristics upon exposure to analytes.

A. Monomer or Oligomer Selection

A variety of polymer sensor chemistries may be utilized in fabricating a wide diversity of sensor families

35 according to the method of the present invention. As will be appreciated by those in the art, while different sensors below list sets of suitable polymers, any of the monomer/polymer combinations may

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be used for any sensor. By way of example, a monomer or oligomer may be selected from any member of the group of condensation polymers derived from such monomers as alcohols, dialcohols, amines, diamines, esters, diesters, carboxylic acids, dicarboxylic acids, diacid chlorides, carbonates, anhydrides, amides, imides, benzoxazoles, benzthiazoles, benzimidazoles, quinozalines, aromatic compounds,

5   including specific polymers such as phenol-formaldehydes, ureaformaldehydes, melamine-formaldehydes, acetyl compounds, lactones, nylons, or polyesters. Alternatively, a monomer may be selected from any member of the group of step-type reaction polymers comprising sulfones, ethers, phenylene oxides, phenylene oxide ethers, Diels-Alder-type reactants, urethanes and arylenes. Monomers may alternatively be selected from any member of the group of vinyl polymers comprising

10   ethylenes, vinyl chlorides, vinylidene chlorides, tetrafluoroethylenes, acrylonitriles, acrylamides, acrylates, methacrylates, acetates, styrenes, including derivatized styrenes such as methyl styrenes, vinyl esters, vinyl pyrrolidones, butylenes and butadienes.

For optical sensors, sensor elements are typically selected based on distinguishable differences in their characteristic optical response signatures when illuminated by excitation light energy in the presence

15   of a target analyte. In fabricating polymer sensor arrays, polymer sensor elements are selected which have characteristic optical response signatures when infiltrated with a reporting dye and illuminated by excitation light energy in the presence of a target analyte. Thus, preferred optical sensor materials for copolymer sensor arrays are selected based on both physical and chemical differences in sensor types which, in combination with a reporter dye compound, produce a characteristic optical response signature

20   in the presence of the analyte when illuminated by excitation light energy.

The following monomer, polymer and copolymer compositions and their derivatives are particularly useful as candidate copolymer materials for combinatorial polymer optical sensors of the present invention: polyethylene glycol, polycaprolactone, polyarylamide, methyl methacrylate [MMA], 2-hydroxyethyl methacrylate, siloxane, dimethylsiloxane, acrlyamide, methylenebisacrylamide [MBA],

25   poly (1,4-butylene) adipate, poly (2,6-dimethyl-1,4-phenyleneoxide) [PDPO], triethoxysilyl-modified polybutadiene (50% in toluene) [PS078.5], diethoxymethylsilyl-modified polybutadiene in toluene [PS078.8], acryloxypropylmethyl- cyclosiloxane [CPS2067], (80-85%) dimethyl-(15-20%) (acryloxypropyl) methylsiloxane copolymer [PS802], poly(acryloxypropyl-methyl)siloxane [PS901.5], (97-98%) dimethyl-(2-3%) (methacryloxypropyl)methylsiloxane copolymer [PS851], poly(acrylonitrile-

30   butadiene-styrene)[PABS], poly(methyl methacrylate), poly(styrene-acrylonitrile 75:25) [PSAN], acryloxypropylmethylsiloxane-dimethylsiloxane copolymer, methylstyrenes, styrenes, acrylic polymers, and methylstyrene divinyl benzene.

Copolymer electrical or electronic sensors which rely on conductance, inductance or resistance as a transduction means for detection of analytes may also be fabricated by the combinatorial polymer

35   synthesis method of the present invention. The following monomer, polymer, and copolymer compositions and their derivatives would be particularly useful as combinatorial polymer candidate

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materials for electrical conductive, inductive or resistive copolymer sensors and sensor arrays of the present invention: metallocene-based ethylenes, ethylene oxides, aromatics, alkyl or alkoxy substituted aromatics, dimethyle siloxanes, alkythiophenes, pyrroles, thiophenes, 3-methyl thiophene, 3,4-dimethyl thiophene, anilines, N-vinyl carbozole, p-phenylene vinylene, acetylenes, styrenes, p-phenylenes,
5   ethylenes, propylenes, dienes, vinyl chlorides, carbonates, vinyl acetates, urethanes, butadienes, acrylates , methacrylates, chloromethylated styrenes, vinylamines, and arsenic pentafluoride or iodine-doped combinatorial polymers comprising polyacetylene, poly-p-phenylene, polypyrrole and polyphenylene sulfide.

Copolymer piezoelectric sensor coatings which have distinguishable analyte sorption, solvation and
10 partition coefficient effects may also be fabricated by the combinatorial polymer synthesis method of the present invention. For example, thin films of the following monomer, polymer, and copolymer compositions and their derivatives are particularly useful as combinatorial polymer synthesis candidate coatings for piezoelectric substrates in piezoelectric-based sensor arrays of the present invention: polyimides, polycarbonates, phthalocyanines, styrene-butadiene-styrene copolymers, cyclophanes,
15 phthalocyanines, thiols, silanes, lipids, nucleic acids, enzymes, antibodies, triethanolamine, quadrol, ethylene dinitrotetraethanol, ascorbic acid, capiscum, L-glutamic acid, pyridoxine, triphenylamine, methyl-trioctylphosphonium dimethyl phosphate, glutathione, NAD, ethylene maleate, triethanolamine, vinyl stearates, collodion, butadiene-acrylonitriles, p-vinyl phenol, hydroxy terminated polybutadiene, vinyl isobutyl ethers, poly vinyl chlorides, caprolactones, caprolactone triols, butadiene methacrylate, methyl
20 methacrylates, polystyrenes, ethylene glycol methyl ether, vinyl carbozoles, abietic acid, octadecyl vinyl ether / maleic anhydride, polyethylenes, ethylcellulose, fluoropolyols, siloxanes, alkylaminopyridyl-substituted polysiloxanes, poly(4-vinyl hexafluorocumyl alcohol), hexafluor-2-propanol-substituted polysiloxanes, polyepichlorohydrin, polybis(cyanopropyl)-siloxanes, polyvinyl tetradecanals, polyisobutylenes, poly(trifluorpropyl)methylsiloxanes, polyethylene maleates, polyvinyl propionates,
25 polyethyl enimines, polyphenyl ethers, docosanol, diglycerols, polydiphenoxyphosphazenes, diethyleneglycol adipate, polychloroprenes, acrylonitrile butadienes, apiezon L, biscyanoallyl polysiloxane, polyepichlorohydrin, pheynlmethyldiphenylsilocone, vinyl-modified trifluoropropylmethylsilicone, tributoxyethyl phosphate, poly(hexyl arylate), poly(2-hydroxyethyl arylate), N-ethyl o,p-toluene sulfonamide, and phenyl ethers.

30 Copolymer sensors which demonstrate affinity for specific carbohydrates or sugars due to either hydrogen bonding or boron bonding, via ester bonds, to sugar molecules are also fabricated by the combinatorial polymer synthests method of the present invention. In one embodiment, such sensors are produced by incorporating a number of different boronic acids, for example anthrylboronic acid or phenylboronic acid, in varying ratios into a polymer or reacting various boronic acids with a polymer,
35 thereby enabling the synthesis of copolymers having unique affinities for selectively binding specific sugars and carbohydrates.

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Sensors made from the following hydrophilic monomer, oligomer, and prepolymer compositions and their derivatives are particularly useful as combinatorial polymer synthesis candidate sensor materials for sugars and carbohydrates in copolymer sensor arrays of the present invention: boronic esters, anthrylboronic acids, anthrylpolyamines, polyethylene glycols, polyalcohols, polyvinyl alcohols,
5   polyethers, polyethylene oxides, polyesters, poly HEMA, polyethylene terepthalate, polyamides, polyacrylamides, nylons, polycarboxylic acids, polyacrylic acid, or polymaleic acid.

Sensors made from copolymers of these compounds rely on affinity of sugars or carbohydrates for either hydrogen bonding or boron bonding with the copolymer. For copolymer sensors which rely on hydrogen bonding, the presence of cis-hydroxyl groups are particularly useful. Carbohydrate and sugar
10   sensors may be employed in sensor arrays which rely on detecting changes in mass due to analyte adsorption or, when combined with fluorescent dyes, they may be used in optical sensor arrays for detecting characteristic optic response signatures in response to analytes. Particularly useful candidate dye materials include fluorescent indicators which may be either incorporated into or conjugated with the copolymer sensor as discussed below.

15   Copolymer sensors which demonstrate affinity for specific metal ions or ion salts due ion-copolymer complex formation may also be fabricated by the combinatorial polymer synthesis method of the present invention. Charged ions having different affinities for metal ions and ion salts are combined with copolymers in varying combinations and ratios to generate unique sensor materials having unique affinities, binding properties and unique response characteristics to analytes. Copolymer sensors made
20   from the following hydrophilic monomer, oligomer, and prepolymer compositions and their derivatives would be particularly useful as candidate combinatorial polymer synthesis sensors for metal ions and ion salts in copolymer sensor arrays of the present invention: polyethylene glycols, polyalcohols, polyvinyl alcohols, polyethers, polyethylene oxides, polyesters, poly HEMA, polyethylene terepthalate, polyamides, polyacrylamides, nylons, polycarboxylic acids, polyacrylic acid, or polymaleic acid.

25   The affinity of candidate copolymer sensors for metal ions and ion salts may be modified by either negatively-charged ligands, such as carboxylic acids, sulfonic acids, phosphates and phosphonates, which attract metals or positively charged ions, or positively charged ligands, such as tetra alkylammonium and phosphonium salts, which attract ion salts or negatively charged ions. Such ligands may further modify the affinity of copolymer sensors by changes in ion coordination, charge repulsion,
30   or steric effects. These ion sensors may be employed in sensor arrays which either rely on detecting changes in mass due to analyte adsorption, or, when combined with fluorescent dyes in optical sensor arrays, detecting characteristic optic response signatures in response to analytes, or, in electrochemical sensor arrays, detecting characteristic potential, current or conductance ranges. Particularly useful candidate dye materials include metallochromic indicators, such azo and triphenylmethane dyes, and
35   fluorescent indicators which may be incorporated into conjugated with the copolymer sensor as discussed below.

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Copolymer sensors which demonstrate characteristic hydrophilic or hydrophobic properties are also fabricated by the combinatorial polymer synthesis method of the present invention for detecting analytes by partitioning of target analytes between the copolymer and a fluid sample medium. With such sensors, a hydrophobic analyte would demonstrate affinity for a hydrophobic copolymer sensor in a

5 hydrophilic fluid medium and hydrophilic analyte would demonstrate affinity for a hydrophilic copolymer sensor in a hydrophobic fluid medium. Alternatively, a hydrophobic copolymer repels a hydrophilic analyte and a hydrophilic copolymer repels a hydrophobic analyte. The relative affinity or repulsion of an analyte for a copolymer sensor leads to partitioning of the analyte between the copolymer and fluid medium and this characteristic may be employed for detecting an analyte in a fluid due to a

10 characteristic physical, electrical, optical or chemical response of the sensor to the partitioned analyte. These analyte-partitioning copolymer sensors may be employed in sensor arrays which either rely on detecting changes in mass due to analyte adsorption from partitioning, or, when combined with fluorescent dyes in optical sensor arrays, detecting characteristic optic response signatures in response to partitioned analytes, or, in electrochemical sensor arrays, detecting characteristic potential, current

15 or conductance changes due to partitioned analytes.

By way of example, copolymer sensors made from hydrophilic monomer, oligomer, and prepolymer compositions and their derivatives are particularly useful as combinatorial polymer synthesis candidate sensors for hydrophilic analytes such as metals, sugars, amino acids, amines, and carboxylic acids. Hydrophilic copolymer sensors made from the following hydrophilic monomer, oligomer, and

20 prepolymer compositions and their derivatives are thus particularly useful as candidate sensors which rely on partitioning approaches: anthrylpolyamines, polyethylene glycols, polyalcohols, polyvinyl alcohols, polyethers, polyethylene oxides, polyesters, poly HEMA, polyethylene terepthalate, polyamides, polyacrylamides, nylons, polycarboxylic acids, polyacrylic acid, or polymaleic acid.

Alternatively, copolymer sensors made from hydrophobic monomer, oligomer, and prepolymer

25 compositions and their derivatives are particularly useful as combinatorial polymer synthesis candidate sensors for hydrophobic analytes such as organic vapors or liquids, alkanes, alkenes, alkynes, alcohols, epoxides, polynuclear aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) and other hydrocarbons. Hydrophobic copolymer sensors made from hydrophobic monomer, oligomer, and prepolymer compositions and their derivatives containing the following functional groups are thus

30 particularly useful as candidate sensors which rely on partitioning approaches: alkanes, alkenes, alkynes, alcohols, epoxides, aromatics, and polycyclic aromatics.

Additionally, copolymer sensors whose characteristic polymer structure creates steric impediments to analyte adsorption provide for partitioning of analytes due to size exclusion effects. By varying ratios of monomers or oligomers to crosslinkers in a prepolymer mixture, a diverse range of steric effects are

35 introduced in a copolymer which provides for partitioning of analytes due to molecular size. By way of example, copolymer sensors based on poly HEMAs, polyacrylamides, as used in electrophoresis gels,

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and cellulose acetates, as employed in dialysis membranes, are particularly useful oligomer candidates for copolymer sensors of the present invention where analytes are detected by size partitioning of target analytes between the copolymer sensor and a fluid sample medium. For vapor sensing, polystyrene with varying amounts of divinylbenzene may be employed to provide size exclusion of vapor analytes.

5   Examples of cross-linkers which are particularly useful for such copolymer sensors include ethylene glycol dimethacrylate (EGDMA) for poly HEMAs, methylene bisacrylamide with polyacrylamides, and divinylbenzene for polystryrene. Such sensors are particularly useful for detecting target analytes such as vapors, macromolecules or oligonucleotides such as proteins and DNA. With such copolymer sensors, the relative size of target analytes provides for partitioning of analytes between the copolymer

10   and fluid medium and this characteristic is employed for detecting such analytes in a fluid due to a characteristic physical, electrical, optical or chemical response of the sensor to the partitioned analyte. These analyte-partitioning copolymer sensors are employed in sensor arrays which either rely on detecting changes in mass due to analyte adsorption from partitioning, or, when combined with fluorescent dyes in optical sensor arrays, detecting characteristic optic response signatures in response

15   to partitioned analytes, or, in electrochemical sensor arrays, detecting characteristic potential, current or conductance changes due to partitioned analytes.

Particularly useful candidate dye materials for either hydrophilic, hydrophobic or steric analyte-partitioning copolymer sensors discussed above include, for hydrophilic sensing environments, metallochromic indicators, such as azo and triphenylmethane dyes, and fluorescent indicators which

20   may be incorporated into or conjugated with copolymer sensors as discussed below. Particularly useful candidate dye materials for such analyte-partitioning copolymer sensors in hydrophobic sensing environments include solvatochromic dyes.

Other polymers and copolymers having distinguishable and characteristic swelling behavior, polarity, conductivity, resistivity, piezoelectric properties or chemical adsorption characteristics are likely

25   candidate materials. Additional sensor matrix candidate materials include copolymers of the compounds listed in Table 7, Table 8 and Table 10 of U.S. Patent 5,512,490 to Walt, et al., which is incorporated herein by reference.

In one alternative embodiment, the physical and chemical properties of copolymer sensor materials are further modified by the addition of plasticizers, including, but not limited to, tritolyl phosphate (TTP),

30   triphenyl phosphate (TTP) or dibutyl phthalate (DBP). In another alternative embodiment, copolymer sensor materials may be further modified by attaching a desirable chemical functional group to the sensor surface or applying either a surface treatment or coating to modify the characteristic sensor response to analytes.

Where the addition of a particular chemical functionality to a copolymer sensor is desirable, examples

35   of surface chemistries which may be attached to sensor surfaces are amines, carboxylic acids,

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aldehydes, aliphatic amines, amides, chloromethyl, hydrazide, hydroxyl, sulfate, sulfonate, aromatic and aromatic amine groups. Additional chemical functionality has been utilized with polymer sensors to produce sensor specificity for certain target analytes or to provide for a characteristic optical response to target analytes. Such functionalities include basic indicator chemistry sensors, enzyme-based
5   sensors, immuno-based sensors and gene-sensors. Examples of such useful functionalities may be found in U.S. Patent Application Serial No. 08/851,203 to Walt, et al., filed on May 5, 1997, which is incorporated herein by reference.

B. Polymerization and Initiators

In fabricating copolymer sensors of the present invention, polymerization of prepolymer mixtures of
10 desired monomer combinations may be achieved by thermal polymerization, condensation polymerization, photoinitiated polymerization, or either crystallization or precipitation from solution followed by annealing.

Thermal polymerization may be utilized either with or without the addition of an initiator. In one embodiment, initiators may be employed to control the rate of thermal polymerization. Since it is often
15 desirable to carry out copolymerization of monomer mixtures at low temperature to prevent side reactions, the selection of thermal initiators is generally restricted to organic peroxides, such as dialkyl peroxides or diacylperoxides, organic hydroperoxides, azo compounds, such as azobisisobutyronitrile, and organometallic reagents, such as silver alkyls. Alternatively, thermal initiation may be accomplished by redox agents, for example, in aqueous solutions, a persulfate salt used in combination with a bisulfite
20 ion reducing agent may form an intermediate sulfate radical ion and subsequent hydroxyl radical initiator. Similar redox reaction initiators may be used by combination of alkyl hydroperoxides and a reducing agent, such as ferrous ion. Additionally, some monomers, such as styrenes, undergo free-radical polymerization when heated or exposed to excitation light energy. Alternatively, anionic or cationic polymerization catalysts may also be employed.

25 In one embodiment, combinatorial polymer synthesis is accomplished by way of condensation polymerization. With this method, no initiator is required and polymerization occurs by way of direct reaction of desired monomers either in the presence or absence of a catalyst to stabilize a metastable intermediate.

In one preferred embodiment, photoinitiated polymerization is utilized. One advantage of
30 photopolymerization is that it offers greater reaction control than thermal polymerization and enables spatial control of local polymerization reactions which can be restricted to regions illuminated by directed light energy. Photopolymerization may be conducted either with or without a specific photosensitizer initiator compound. For example, in the absence of a specific photosensitizer, many candidate monomer materials that can undergo chain reaction polymerization are susceptible to
35 photopolymerization since the absorption of light produces free radicals or ions. Examples of such

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compounds are unsaturated monomers including, but not limited to, vinyl alkyl ketones, vinyl bromides, styrene and derivatives, methyl methacrylate and isobutylene.

In one alternative embodiment, a photosensitizer must be added to the prepolymer mixture of monomers for photopolymerization of the copolymer. Photosensitizers are compounds that absorb light

5   in a desired region of the spectrum, typically ultraviolet or visible light, and subsequently dissociate into free radicals or transfer absorbed energy directly to a monomer. While some thermal initiators, such as azo compounds and peroxides are also photosensitizers, many alternative initiators are used as photosensitizers even though they do not dissociate thermally at useful temperatures. Examples of particularly useful photosensitizers are carbonyl compounds, such as acetone, biacetyl benzophenone

10   benzoin, or α-chloroacetone, condensed ring aromatics, such as anthracene, peroxides, such as t-butyl peroxide or hydrogen peroxide, organic sulfides, such as diphenyl disulfide or dibenzoyl disulfide, azo compounds, such as azoisopropane, azobisisobutyronitrile or aryldiazonium salts, halogen-containing compounds, such as chlorine, chloroform, carbon tetrachloride, bromotrichloromethane, bromoform or bromine, metal carbonyls, such as manganese pentcarbonyl and carbon tetrachloride or rhenium

15   pentacarbonyl and carbon tetrachloride, and inorganic ions, such as $FeOH^{+2}$ or $FeCl_4$. In one preferred embodiment, benzoin ethyl ether initiator was utilized.

C. Dye Selection

For optical sensors which rely on light absorption and emission, the selection of chemical dye indicators is also important to the design and performance of the combinatorial copolymer sensors and sensor

20   arrays of the present invention. An important characteristic of candidate dye materials for optical sensor elements is that they can be readily incorporated into copolymer sensor matrices and that, once incorporated into a copolymer matrix, their optical response characteristics are modified by the localized polymer microenvironment. In one embodiment, at least one dye is incorporated into the copolymer sensor matrix by way of entrapment. In an alternative embodiment, two or more dyes may be

25   incorporated into the copolymer sensor matrix and peak intensity ratio for the dye pair may be used for providing a characteristic optical response signature for target analytes. In an alternative embodiment, conjugated dyes, such as acryloyl fluorescein and others, may be utilized where it is desirable to incorporate the dye directly into the copolymer sensor material by way of covalent bonding. Particularly useful references for selection of candidate dyes such as metallochromic indicators, including azo and

30   triphenylmethane dyes, and fluorescent indicators, which may be either incorporated or conjugated with copolymer sensors of the present invention, is Indicators [E. Bishop (ed.), Pergamon Press (New York 1972)], and the 6th Edition of Molecular Probes Handbook by Richard P. Haugland, both of which are incorporated herein by reference.

While the reporter dye may be either a chromophore-type or a fluorophore-type, a fluorescent dye is

35   preferred because the strength of the fluorescent signal typically provides a better signal-to-noise ratio and improves detection of target analytes. In the most preferred embodiment, polarity-sensitive dyes

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or solvatochromic dyes are utilized. Solvatochromic dyes are dyes whose absorption or emission spectra are sensitive to and altered by the polarity of their surrounding environment. Typically, these dyes exhibit a shift in peak emission wavelength due to a change in local polarity. Polarity changes which cause such wavelength shifts can be introduced by the copolymerized matrix used for a particular

5   sensor family, by the presence of a target analyte, or by the combination of the copolymer matrix and analyte interaction with the dye. The change in polarity creates a characteristic optical response signature which is useful for detecting specific target analytes. One preferred solvatochromic dye is Nile Red, available from Eastman Kodak (Rochester, NY). Nile Red exhibits large shifts in its emission wavelength peak with changes in the local environment polarity. In addition, Nile Red is soluble in a wide

10   range of solvents, is photochemically stable, and has a relatively strong fluorescence peak. Alternatively, other solvatochromic dyes such as Prodan, 6-propionyl-2-(N,N-dimethylamino)napthalene, or Acrylodan, 6-acryloyl(dimethylamino)napthalene, available from Molecular Probes (Eugene, OR), may be employed. Additional dyes which are conventionally known in the art and may be used as dyes in the present invention are those found in U.S. Patent 5,512,490 to Walt, et al., of which Table 3, Table

15   4, Table 5, Table 6 and Table 11 are incorporated herein by reference.

Diverse families and types of optical sensor elements may be fabricated as sensors and sensor arrays of the present invention by incorporating reporter dyes, such as metallochromic indicators, fluorescent indicators, or solvatochromic dyes, within various copolymer matrices of varying monomer compositions. By incorporating such dyes in sensor elements made from different copolymer matrices of varying

20   polarity, hydrophobicity, pore size, flexibility and swelling tendency, unique sensors are produced that react differently with molecules of individual analytes, giving rise to distinguishable and characteristic fluorescence responses when exposed to target analytes.

D. Sensor Configuration

The sensors of the present invention can be configured in a variety of ways, including, but not limited to,

25   polymerization of the copolymer sensors directly on a substrate, polymerization of the copolymer sensors onto the surface of microspheres, and polymerization of the copolymer sensors into microspheres; the latter two embodiments then distribute the microspheres on the substrate.

In a preferred embodiment, the combinatorial copolymer sensors are polymerized directly onto discrete sites on a substrate. By "discrete sites" is meant individual sites or loci on a substrate. The sites may

30   be a pattern, i.e. a regular design or configuration, or randomly distributed. A preferred embodiment utilizes a regular pattern of sites such that the sites may be addressed in the X-Y coordinate plane. A pattern in this sense includes a repeating unit cell, preferably one that allows a high density of sites on the substrate. However, it should be noted that these sites may not be discrete sites. That is, it is possible to use a uniform surface of adhesive or chemical functionalities, for example, that allows the

35   association of copolymers, or in the case where microspheres are used, for association of the beads at any position. That is, the surface of the substrate is modified to allow association of the copolymers

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(microspheres) at individual sites, whether or not those sites are contiguous or non-contiguous with other sites. Thus, the surface of the substrate may be modified such that discrete sites are formed that can only have a single associated copolymer, or alternatively, the surface of the substrate is modified and copolymers may go down anywhere, but they end up at discrete sites.

5   By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites. In a preferred embodiment such sites are appropriate for the attachment or association of beads. The substrate also is amenable to at least one detection method. As will be appreciated by those in the art, the number of possible substrates is very large. Possible substrates include, but are not limited to, glass and modified or functionalized glass, plastics

10   (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyurethanes, Teflon, etc.), polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses, plastics, optical fiber bundles, and a variety of other polymers. Also, metals, ceramics, graphites, semiconductors, and composites fabricated from such materials are useful as substrates. In

15   general, the substrates allow optical detection and do not themselves appreciably fluoresce.

Such substrate materials may be preconditioned or functionalized using conventionally known surface treatments so as to improve sensor adhesion or to immobilize sensors on the substrate. The ultimate choice of a preferred substrate material depends on the actual sensor format and transduction mechanism as well as the targeted sensing environment and application.

20   For electrical sensors, suitable substrates include conventional dielectric, magnetic, conducting, and semiconducting materials as well as electrical components such as electrodes, capacitors, resistors, diodes and transistors. For mass sensitive sensors, potential substrates include any piezoelectric materials or devices such as quartz crystal microbalances, microcantilevers, and surface acoustic wave devices. For surface plasmon resonance sensors, potential substrates include thin reflective surface

25   films comprised of gold, silver, chrome, nickel or any other highly reflective material. For optical sensors, transparent, opaque or reflective substrates may be employed depending on the orientation of the sensor relative to the analyte medium and incident excitation light source, and detection means. In one preferred embodiment, an individual fiber optic strand or a fiber optic array, comprising either a fiber optic bundle, or preformed, unitary fiber optic array, or an imaging fiber, comprised of a plurality of

30   individual fibers, may be used as an optical sensor substrate where the excitation light and optical response of the sensor are conveyed to and from sensors which are immobilized on one end of the fiber strand or fiber array.

Accordingly, a preferred embodiment utilizes fiber optic sensors. This is particularly useful when photoinitiation is done. As one of ordinary skill in the art appreciates, when using fiber optic bundles, light

35   is transmitted through individual fibers where it contacts the copolymer thereby initiating polymerization.

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From one to all of the individual fibers of a fiber optic bundle are illuminated. In an alternative embodiment, only a subset of fibers are illuminated.

In a preferred embodiment, the sensors of the invention comprise microspheres. By "microspheres" or "beads" or "particles" or grammatical equivalents herein is meant small discrete particles. The

5   composition of the beads will vary, depending on the configuration of the array and the copolymers used, as well as the method of synthesis. Suitable bead compositions include those used in peptide, nucleic acid and organic moiety synthesis, including, but not limited to, plastics, ceramics, glass, polystyrene, methylstyrene, acrylic polymers, paramagnetic materials, thoria sol, carbon graphite, titanium dioxide, latex or cross-linked dextrans such as Sepharose, cellulose, nylon, cross-linked micelles and Teflon

10   may all be used. *"Microsphere Detection Guide"* from Bangs Laboratories, Fishers IN is a helpful guide.

The beads need not be spherical; irregular particles may be used. In addition, the beads may be porous, thus increasing the surface area of the bead available for exposure to the target analyte or for either bioactive agent attachment or IBL attachment. The bead sizes range from nanometers, i.e. 100 nm, to millimeters, i.e. 1 mm, with beads from about 0.2 micron to about 200 microns being preferred,

15   and from about 0.5 to about 5 micron being particularly preferred, although in some embodiments smaller beads may be used.

It should be noted that a key component of the invention is the use of a substrate/bead pairing that allows the association or attachment of the beads at discrete sites on the surface of the substrate, such that the beads do not move during the course of the assay.

20   In a preferred embodiment, the copolymers are polymerized onto the surface of the beads. This may be desirable to increase the surface area, particularly when porous beads are used. Accordingly, each bead is contacted with a different combination of copolymers which are then polymerized onto the surface of the bead. This results in the formation of a population of heteregenous bead sensors. Alternatively, each bead is contacted with the same combination of copolymers which are polymerized

25   to create a homogenous population of bead sensors.

In a preferred embodiment, the copolymers are polymerized into beads that are then distributed on a surface. For example, any of the copolymer combinations are used to fabricate beads in accordance with methods known to those of ordinary skill in the art.

E. Substrate Selection

30   The combinatorial copolymer sensors of the present invention may be deployed on virtually any solid substrate material as either discrete individual sensors or as a plurality of sensor elements in a sensor array. Suitable substrate materials include, but are not necessarily limited to metals, ceramics, glasses, plastics, polymers, graphites, semiconductors, and composites fabricated from such materials. Such

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substrate materials are preconditioned or functionalized using conventionally known surface treatments so as to improve sensor adhesion or to immobilize sensors on the substrate. The ultimate choice of a preferred substrate material depends on the actual sensor format and transduction mechanism as well as the targeted sensing environment and application.

5   For electrical sensors, suitable substrates include conventional dielectric, magnetic, conducting, and semiconducting materials as well as electrical components such as electrodes, capacitors, resistors, diodes and transistors. For mass sensitive sensors, potential substrates include any piezoelectric materials or devices such as quartz crystal microbalances, microcantilevers, and surface acoustic wave devices. For surface plasmon resonance sensors, potential substrates include thin reflective surface

10   films comprised of gold, silver, chrome, nickel or any other highly reflective material. For optical sensors, transparent, opaque or reflective substrates are employed depending on the orientation of the sensor relative to the analyte medium and incident excitation light source, and detection means. In one preferred embodiment, an individual fiber optic strand or a fiber optic array, comprising either a fiber optic bundle, or preformed, unitary fiber optic array, or an imaging fiber, comprised of a plurality of

15   individual fibers, may be used as an optical sensor substrate where the excitation light and optical response of the sensor are conveyed to and from sensors which are immobilized on one end of the fiber strand or fiber array.

2. Sensor and Sensor Array Fabrication

A. Discrete Copolymer Sensors

20   For fabrication of sensor arrays comprising a selection of discrete copolymer sensors formed by the method of the present invention, at least two or more monomers or prepolymers are employed at preselected monomer or prepolymer ratios so as to provide a broad range of monomer or prepolymer compositions. In one embodiment, an (80-85%) dimethyl-(15-20%)(acryloxypropyl)methylsiloxane copolymer, commonly known as PS802 and available from Gelest Inc. (Tullytown, PA), and methyl

25   methacrylate, commonly known as MMA and available from Aldrich (Milwaukee, WI) were selected as example monomers for copolymerization. Typically, separate solutions of monomer or prepolymer mixtures are prepared representing a range of monomer or prepolymer composition ratios. In one embodiment, the range of at least one monomer or prepolymer composition varies from between O to 100% of the monomer. In an alternative embodiment, at least one composition ranges from between

30   0 to 50%. In alternative embodiments, at least one composition ranges from between 0 to 25%. Other, more narrow, composition ranges may also be employed.

The monomer or prepolymer mixtures are copolymerized on any suitable substrate (or into beads) by either a photoinitiated polymerization or a conventional thermal polymerization method. With either method, an initiator solution and dye solution are added to the prepolymer or monomer mixture either

35   prior to, during or after polymerization for the purpose of minimizing exposure and controlling reaction with the initiator. In a preferred embodiment, a solvatochromic dye is utilized. In one preferred

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embodiment, a solution of Nile Red in chloroform is used as the dye solution and a solution of benzoin ethyl ether in toluene is used as a photoinitiator.

In one preferred embodiment, each of the monomer mixture-dye-initiator solutions is individually photopolymerized and photodeposited onto the end of a preformed fiber optic array or imaging fiber.

5   In this embodiment, a distal end of the fiber is either immersed directly in a prepolymer solution or, alternatively, a polymerization solution is applied to a distal end of the fiber. Using conventional optical train components, a series of pinholes, lenses and objectives focuses an ultraviolet light beam on pre-selected fiber end faces of the fiber array. The light is transmitted down the length of the individual fibers and exits at the distal end faces of the fibers at the predetermined fiber locations in the array. The

10   photopolymerization of individual prepolymer monomer mixtures is thereby initiated at designated regions across the distal end surface of the fiber array. In this manner, the photodeposition method and system described herein provides for the formation of a plurality of discrete sensor elements and sensing regions across the distal end surface of the fiber array.

Typically, the size of the photopolymerized deposit may be controlled by the polymerization reaction time

15   and polymerization rate. The reaction rate is influenced by polymerization light intensity, concentrations of photoinitiator and monomers and choice of initiator and monomer composition. The size of photodeposits can be controlled over a diameter range from several microns, covering the end of an individual fiber strand in the fiber optic array, to over 100 microns, covering adjacent multiple fiber strands. In one embodiment, each of the prepolymer monomer mixture solutions was polymerized for

20   5 seconds, resulting in approximately 45μm-diameter polymer hemispheres or cones attached to the distal end of a fiber optic array. Multiple deposits of each monomer combination mixture may be polymerized at various fiber locations in the fiber optic array. By forming multiple deposits of each monomer combination, a sensor array having a unique arrangement of sensing elements is thereby produced.

25   B. Copolymer Gradient Sensors

With the copolymer gradient sensors and gradient sensor arrays of the present invention, the elements of the sensor array are no longer discrete individual polymer deposits, but rather user-defined, regions-of-interest (ROI), outlining specific portions of the gradient polymer deposit to be monitored. Following the collection of a sequence of fluorescence image frames with a CCD camera, one can use

30   standard drawing tools included in commercial image processing software packages to either manually or automatically select areas of the gradient sensor images from which to measure fluorescence. Examples of such pre-selected areas are shown in Figs. 5a-b and Figs. 8a-b.

Typically, copolymer gradient sensors are prepared by photodepositing a polymer strip from a prepolymer mixture having a time-varying monomer composition. In a typical procedure, the distal end

35   of a fiber optic array is immersed in a stirred first solution comprising at least one monomer and a

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photoinitiator. For photodeposition of the copolymer, a UV excitation light beam is scanned across the proximal end of the fiber array at a predetermined scanning rate while a second solution, comprising at least one additional monomer and a photoinitiator, is simultaneously added and mixed with the first solution. Generally, both solutions are flushed with nitrogen prior to the mixing photopolymerization of

5   the monomers. This photodeposition method produces a copolymerized polymer strip having a continuously varying structure and composition over its entire length due to the monomer composition variation imposed in the prepolymer mixture during polymerization of the solution. After photodeposition of the copolymer gradient strip, the deposit is soaked in a dye solution comprising a solvent and dye, rinsed, and dried overnight. In a preferred embodiment, the copolymer gradient sensors are contacted

10   with a dye solution comprising solvatochromic dye dissolved in an organic solvent that will swell the copolymer gradient sensor matrices. In a subsequent step, the gradient sensors are washed to remove excess dye. Typically, the sensors are washed in water, methanol, or any suitable solvent that does not swell the copolymer matrix, but in which the dyes are still soluble. This allows the residual dye to be rinsed off without rinsing the dye out of the sensor matrices.

15   C. Sensor Array Fabrication
The copolymer sensor elements of the present invention may be deposited on any suitable substrate materials to form a sensor or sensor array. A wide diversity of materials may be utilized as substrates. While the substrate may be formed from any suitable organic or inorganic materials, the substrate should be chemically inert to the sensor elements, target analytes and any analyte solvent matrix.

20   Examples of suitable substrate materials include glass, ceramics, plastics, polymeric materials, metals or composite materials. The size, shape, and configuration of the substrate can be adapted to meet the requirements of a particular sensing application or environment. A principal requirement in selecting a suitable substrate dimension and configuration is that the substrate should provide for access of target analytes to the copolymer sensor elements. The variety of substrate configurations and shapes

25   envisioned by the sensor of the present invention includes fibers, rods, plates, spheres, or any curved, rectilinear or irregular surfaces as well as piezoelectric substrates and electrodes.

In one embodiment for optical sensor arrays, a sensor array 100, comprising a plurality of individual sensors, are located adjacent to or attached to a distal end 212 of a optical fiber bundle 202. In one preferred embodiment, discrete sensors or gradient sensors are deposited directly on a distal end 212

30   of a fiber bundle 202. It is important that the sensors and sensor array are located proximate to the distal end 212 of the fiber optic bundle 202 to ensure that the light returning in each discrete optical fiber 252 predominantly originates from only a single sensor or a portion of a gradient sensor. This feature is necessary to enable the interrogation of the optical signature of individual sensors or portions of gradient sensors within the sensor array 100, and to provide for the summing of individual sensor responses

35   within each sensor type for reducing signal to noise and improving signal enhancement. In this embodiment, the sensor adhesion or affixing technique must not chemically insulate the sensor from the analyte or otherwise interfere with the optical measurement.

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In one preferred embodiment, each individual optical fiber 252 of the fiber bundle 202 conveys light from a single discrete sensor or a portion of a gradient sensor.

Consequently, by imaging the light emanating from a proximal end 214 of the fiber bundle 202 onto a detector array such as a CCD camera, the optical signatures of discrete sensors or portions of gradient
5   sensors are individually interrogatable.

Where a fiber optic array is employed as a sensor array 100 substrate, the fiber optic array may be a either a conventional imaging fiber, comprising a preformed, unitary array of a plurality of prefused individual fibers, or a conventional fiber bundle comprised of a plurality of discrete individual fibers, where the individual fiber strands are disposed coaxially along their lengths. In one preferred
10   embodiment, the individual fibers have a cladding for reducing optical crosstalk between fibers in the array. While a fiber optic array will typically comprise thousands of discrete fibers of uniform diameter, alternative embodiments can provide for variations in both the number of fibers and range of fiber diameters within an array. Individual fiber diameters of the fiber optic array may range from approximately 1 to 500 um. Individual fibers may have either a circular or non-circular cross-section and
15   mixtures of cross-sections may be employed in an array. The individual fibers of the array may be arranged either in coherent or incoherent manner. For the sensor arrays described in Examples 1-3, commercial image guides were used as fiber optic array substrates for photodeposition of the copolymer sensor elements. These image guides, available from Galileo Electro-Optics Corporation (Sturbridge, MA), typically comprise approximately 6000 optical fibers, each fiber having a 2-4 µm diameter, packed
20   together m a coherent fashion such that spatial position is maintained from one end of the fiber to the other.

Prior to photodeposition of copolymer sensor elements, the distal end surface of the fiber optic array is typically cut to length, polished and prepared for photodeposition. Generally, both the proximal and distal ends of the fiber array bundle are successively polished on 12 µm, 9 µm, 3 µm, 1 µm, and 0.3 µm
25   lapping films. Subsequently, the ends may be inspected for scratches on a conventional microscope. The fiber is typically rinsed in water and acetone and ultrasonically cleaned for several minutes to remove any polishing residue. The fiber is then allowed to dry prior to a silanization treatment.

When the sensor comprises beads, the substrate can be prepared in a variety of ways. In a preferred embodiment, when the sensor comprises beads, the substrate may be prepared to include discrete
30   sites. In a preferred embodiment, the surface of the substrate is modified to contain wells, i.e. depressions in the surface of the substrate. This may be done as is generally known in the art using a variety of techniques, including, but not limited to, photolithography, stamping techniques, molding techniques and microetching techniques. As will be appreciated by those in the art, the technique used will depend on the composition and shape of the substrate.

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In a preferred embodiment, physical alterations are made in a surface of the substrate to produce the sites. In a preferred embodiment, the substrate is a fiber optic bundle and the surface of the substrate is a terminal end of the fiber bundle, as is generally described in 08/818,199 and 09/151,877, both of which are hereby expressly incorporated by reference. In this embodiment, wells are made in a terminal

5 or distal end of a fiber optic bundle comprising individual fibers. In this embodiment, the cores of the individual fibers are etched, with respect to the cladding, such that small wells or depressions are formed at one end of the fibers. The required depth of the wells will depend on the size of the beads to be added to the wells.

Generally in this embodiment, the microspheres are non-covalently associated in the wells, although

10 the wells may additionally be chemically functionalized as is generally described below, cross-linking agents may be used, or a physical barrier may be used, i.e. a film or membrane over the beads.

In a preferred embodiment, the surface of the substrate is modified to contain chemically modified sites, that can be used to associate, either covalently or non-covalently, the microspheres of the invention to the discrete sites or locations on the substrate. "Chemically modified sites" in this context includes, but

15 is not limited to, the addition of a pattern of chemical functional groups including amino groups, carboxy groups, oxo groups and thiol groups, that can be used to covalently attach microspheres, which generally also contain corresponding reactive functional groups; the addition of a pattern of adhesive that can be used to bind the microspheres (either by prior chemical functionalization for the addition of the adhesive or direct addition of the adhesive); the addition of a pattern of charged groups (similar to

20 the chemical functionalities) for the electrostatic association of the microspheres, i.e. when the microspheres comprise charged groups opposite to the sites; the addition of a pattern of chemical functional groups that renders the sites differentially hydrophobic or hydrophilic, such that the addition of similarly hydrophobic or hydrophilic microspheres under suitable experimental conditions will result in association of the microspheres to the sites on the basis of hydroaffinity. For example, the use of

25 hydrophobic sites with hydrophobic beads, in an aqueous system, drives the association of the beads preferentially onto the sites. As outlined above, "pattern" in this sense includes the use of a uniform treatment of the surface to allow association of the beads at discrete sites, as well as treatment of the surface resulting in discrete sites. As will be appreciated by those in the art, this may be accomplished in a variety of ways.

30 The copolymer sensor elements may be deposited with or without pretreating the fiber optic array end surface. In a preferred embodiment, the distal end surface of a fiber optic array bundle is typically activated with a silanizing reagent for improving the adhesion of the copolymer sensor deposit to the fiber array. For photodeposition of copolymer sensor elements, fiber array ends are typically treated by immersion in a 10% solution of 3(trimethoxysilyl)propyl-methacrylate in acetone, rinsed and cured in the

35 absence of light for approximately one hour at room temperature. For thermal polymerization of copolymer sensor elements, the distal end of a fiber array is typically treated with a 2% solution of

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noctadecyltriethoxysilane in 95% ethanol-acetic acid mixture at pH 4.5 for two minutes. The fiber end is rinsed with ethanol and cured at 100°C for 10 minutes.

For photodeposition of the copolymer sensor elements, in one embodiment the distal end of the fiber array may either be immersed in a prepolymer solution comprising a photoinitiator, dye, and at least two

5   monomers that can be photopolymerized. Alternatively, the prepolymer solution may be applied to the distal end of the fiber. In one embodiment, a solvent may be utilized for improving the solubility of reagents in the prepolymer mixture. The proximal end of the fiber array is illuminated with excitation light at a predetermined intensity for a specified time depending on the composition and reactivity of the prepolymer solution. The time and intensity of light exposure is established by shutter and radiometer

10 settings on the photodeposition system. In one embodiment, the fiber is illuminated while the distal end is immersed in the prepolymer solution. In an alternative embodiment, the fiber is dipped in the solution, removed and then illuminated. As a result of controlled illumination, a photodeposit of appropriate size and area is formed on the distal end of the fiber array. The photodeposition conditions may be controlled to produce precisely scaled, individual deposits on the end of each fiber in the fiber array or,

15 alternatively, to produce a larger deposit which covers the ends of multiple adjacent fibers. Typically, for gradient sensor fabrication, a larger deposit encompassing multiple fiber ends is employed so that multiple regions-of interest within the gradient sensor can be optically coupled to individual fibers in the fiber array.

In an alternative embodiment, dye may be incorporated into individual copolymer sensor elements or

20 a sensor array following photodeposition of the copolymer matrix. With this embodiment, the individual sensors or, alternatively, the entire sensor array may be exposed to a dye solution comprised of a dye and appropriate solvent. For example, the copolymer sensors or sensor array may be contacted with a dye solution comprising solvatochromic dye dissolved in an organic solvent that will swell the copolymer gradient sensor matrices. In a subsequent step, the gradient sensors are washed to remove

25 excess dye. Typically, the sensors are washed in water, methanol, or any suitable solvent that does not swell the copolymer matrix, but in which the dyes are still soluble. This allows the residual dye to be rinsed off without rinsing the dye out of the sensor matrices.

In one alternative embodiment, a chemical moiety or functional group may be attached to copolymer sensor elements either prior to the dye incorporation step or following removal of excess dye after the

30 incorporation step.

D. Photodeposition System

Photodeposition of the discrete copolymer sensors and gradient copolymer sensors was performed with a modified commercial photodeposition system. A schematic diagram of the photodeposition system 50 is shown in Fig. 1. A Novacure ultraviolet spot-cure photodeposition system, available from EFOS

35 (Ontario, Canada) was modified for the photopolymerization copolymer depositions of Examples 1

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through 3. A 100 Watt short-arc mercury-xenon lamp 105 with a liquid light guide was employed as a source for uv excitation light. The spot-cure system has a built-in radiometer for controlling the light intensity and an electronic shutter 110 for controlling the polymerization time. Typically, excitation light at 330 nm wavelength and approximately $1100mW/cm^2$ intensity was utilized. Other wavelengths and

5   intensities may be utilized to match specific monomer compositions and concentrations. The excitation light was collimated with a 50 mm focal length lens 115, passed through a series of neutral density filters 140, excitation filters 145, a 100 um pinhole 120, and focused onto the proximal end 214 of a fiber optic bundle 202 with a 15x reflecting microscope objective 125. A conventional fiber chuck 130 and a Spindler and Hoyer (Milford, MA) coarse xy-micropositioner 135 was utilized for securing, positioning and

10 focusing the proximal end surface 214 of the fiber optic bundle 202 with respect to the excitation light beam. The xy-positioner 135 provided 360° rotation, allowing for precise positioning of the proximal end surface 214 of the fiber bundle 202. The distal end 212 of the fiber bundle 202 was immersed in the prepolymer solution 150 of monomers, photoinitiator and dye.

For copolymer gradient sensor depositions, a Burleigh Instruments (Fishers, NY) Model No. 6000 ULN

15 Inchworm PZT nanopositioner 136 was utilized in conjunction with the coarse xy-positioner 135 and fiber chuck 130 for securing, positioning, and translating selected regions of the proximal end 214 of the fiber optic array 202 across the optical axis of a focussed light beam. The focussed light beam remained stationary while the proximal end surface 214 of the fiber bundle 202 was translated across the beam focal point using the nanopositioner 136. A hand-held controller provided precise remote control of the

20 nanopositioner 136 scanning. Scanning speed was adjusted to accommodate either the polymerization rate or addition mixing rate of the monomers in the prepolymer solution 150 at the distal end 212 of the fiber bundle 202. In an alternate embodiment, the focussed light beam may be translated across a stationary proximal end 214 of the fiber bundle 202. While this modification of the photodeposition apparatus 50 provides for a single copolymer gradient sensor to be deposited at a time, it is anticipated

25 that multiple copolymer gradient sensors can be fabricated either sequentially, by repositioning the proximal end surface 214 of the fiber optic array 202 with respect to the optical axis of the light beam, or simultaneously, using modifications of the optical train and apparatus to provide for multiple excitation light beams to be focused simultaneously on multiple areas of the proximal end surface 214 of the fiber array 202.

30 **3. Experimental Measurements**

A. Sensing Apparatus and System:

Characteristic temporal optical response data measurements of discrete copolymer sensors and selected regions of copolymer gradient sensors responses to specific vapor analytes and excitation light energy were made according to the general method, apparatus and instrumentation disclosed by White,

35 et al., *Anal. Chem.* 68:2191-2202(1996), hereby expressly incorporated by reference.

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In Fig. 2, a schematic block diagram illustrates the experimental apparatus and instrumentation 200 used for data measurements obtained with fiber optic sensor arrays of the present invention. In a typical measurement, a proximal end 214 of a fiber optic array 202 was placed in a fiber chuck 210 and secured for viewing with an optical instrumentation system utilizing a modified Olympus microscope 220.

5   A sensor array 100 comprising a plurality of discrete sensors or gradient sensors disposed on a distal end 212 of the bundle 202 was exposed to a fluid sample containing analytes to be detected. In an alternative embodiment, a microscope 220 slide platform and slide clamp was used for viewing and positioning sensor array substrates, such as glass plates, slides or cover slips. The microscope 220 was equipped with an epi-illuminator (λ_{ex} 540 nm, λ_{em} 590 nm, Zeiss 2.5 objective, 0.8 NA) and Olympus

10   20x and 40x and Zeiss 100x objectives. An Omega 560 DCRP dichroic mirror 230 was used to direct excitation light energy from a 75W Xenon arc lamp 240, via the fiber bundle 202, to the sensor array 100 at the distal end 212 of the bundle 202. The dichroic mirror 230 was capable of reflecting light at shorter wavelengths (<560 nm) and transmitting light at longer wavelengths (>595 nm). The excitation light energy emanating from the arc lamp 240 was filtered by an Omega 535 BP40 integrated excitation light

15   filter/shutter 250. The characteristic optical response signature of the array 100, emitted by individual sensor elements or portions of gradient sensors upon illumination by excitation light in the presence of analytes, was transmitted via the fiber bundle 202 and dichroic mirror 230 to a CCD frame transfer camera 270. The light energy emitted from the sensors or portions of gradient sensor of the sensor array 100 was filtered with an Omega 640 BP20 integrated emitted light filter/shutter 260 prior to the

20   CCD frame transfer camera 270. In one embodiment a TE/CCD-512EFT Princeton Instruments (Trenton, NJ) 512 x 512 frame transfer CCD camera 236 was utilized for capturing frames of characteristic fluorescence response images of sensor elements in the sensor array 100.

A 8100AV Macintosh Power PC desktop computer 238 with a Princeton Instruments NUBus camera interface card was employed for data acquisition and processing images acquired by the CCD camera

25   236. Experiments generally consisted of collecting video camera frames of fluorescence response images and recording the images with the CCD frame transfer camera 270. Captured images are then conveyed to the camera interface card in the computer system 280. Depending on the response time of sensor elements, the duration of the analyte-sensor array interaction and the rise and decay of sensor responses to an analyte exposure, camera frame rates and measurement times are selected for

30   collection of a suitable number of data points. Frame capture times typically range between 80 to 250 ms/frame.

B. Sensor Response Testing:

A conventional air dilution olfactometer and vacuum-controlled vapor delivery system 290, as commonly known and used in olfactory research and described in Kauer, et al., *J. Physiol.* 272:495-516 (1977) and

35   White, et al., *Anal. Chem.* 68(13):2191 (1996) was used to apply controlled pulses of analyte vapor and air carrier gas to either a sensor substrate or the distal end 212 of a fiber optic sensor array 100 containing an array of discrete sensors or portions of gradient sensors.

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To produce a saturated vapor sample, in one embodiment, a stream of air carrier gas was passed through a 5 ml cartridge containing filter paper saturated with an analyte. In an alternative embodiment, the carrier gas was passed through a 100 ml sample of analyte contained in a 250 ml Erlenmeyer flask. Analyte dilutions were produced by adjusting the relative flow rates of saturated vapor and clean carrier

5   gas streams. Typically, a flow rate of 150 ml/min is used for the combined gas flow to the sensor array. At this flow rate, a 3 second pulse would deliver approximately 7.5 ml of analyte vapor with carrier gas. In general, depending on the analyte vapor pressure and dilution factor. vapor pulses contain between $1.6x10^{-6}$ to $4.0x10^{-5}$ mol of analyte.

For 60 frame measurements, the vapor pulse is typically delivered during the 11th through 30th frame,

10   commencing on the 11th frame. For 40 frame measurements, the vapor pulse is typically delivered during the 6th through 20th frame, commencing on the 6th frame. The duration of the vapor pulse varies with the specific frame rate utilized and typically ranges between 1 to 4 seconds. Baseline control measurements are performed with high purity, Ultra Zero grade air. The air pulse measurements are performed to account for any sensor responses due to the vapor carrier gas.

15   The sensor arrays of Example 1 and Example 2 were tested by exposing each array to a number of representative analyte vapors using the vapor delivery system and imaging system described herein. In typical sensor response tests for each analyte, a total of 40 time points were collected over 4 seconds, with a vapor pulse duration of 1s. The CCD camera integration time was set to 25ms, and pixels were binned 5 x 5 to enhance signal and reduce readout times. For testing these sensors, the sensor array

20   was typically illuminated with 535nm light, and emission was monitored at 629nm using a 20 nm band pass, liquid crystal tunable filter (Cambridge Research Instruments, Cambridge, MA).

4. Data processing and Analysis
A. Data Processing
Following the collection of a temporal series of sensor element or sensor array images, segments are

25   typically drawn, using IPLab image processing software (Signal Analytics, Vienna, VA), over each pixel or groups of pixels which correspond to an individual fiber where the fiber is coupled to either a discrete sensor or a portion of a gradient sensor at its distal end. The mean fluorescence intensity is measured for each one of these segments in each frame in the sequence. This is done for both the vapor pulse responses and the baseline air pulse responses. Averages of multiple runs of each may be performed

30   to improve data quality where needed. The air pulse data are then subtracted from the vapor pulse data to subtract the background due to air alone. The resulting data can be plotted to yield temporal intensity responses for all sensor types of interest.

All data manipulation is performed within the IPLab program environment using simple scripts written by the operator which call imbedded image or data processing functions. These scripts and routines

35   consist of a data collection portion and a data analysis portion.

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In the data collection portion, there are three segments or loops as follows:

Loop 1. This establishes the baseline fluorescence of each sensor. This loop can be shortened or extended to adjust to slower or faster response times of specific sensors or sensor arrays to certain analytes. In typical measurement runs, this loop is set between 5 to 20 frames.

5   Loop 2. This is the vapor exposure loop. A vapor pulse is applied just before this loop starts by way of a script command that sends a 5 volt pulse to an attached solenoid valve which switches a vacuum line off, thereby allowing a vapor sample to emit from the end of a nozzle. Typically, this loop is 5-30 frames in duration with a 20 frame duration most common.

Loop 3. This is a sensor recovery loop. Another 5 volt trigger pulse is sent to a solenoid which switches

10   back to its initial position, causing the vapor delivery system 290 to resume collection of the solvent vapor and carry it off to waste. Typically, this loop is of 30 frames duration in a 60 frame measurement and 20 frames duration in a 40 frame measurement.

For each loop, the frame rate and number of frames captured are adjusted to capture an appropriate number of data points for a sensor-analyte interaction. Loop duration and measurement times can be

15   adjusted for each measurement to accommodate the response time of sensor elements, the duration of the analyte-sensor array interaction and the rise and decay of sensor responses to an analyte exposure.

B. Data Analysis

In the data analysis portion, pre-selected segments taken from a previously collected "focus" image are

20   transferred to the sequence of images collected. These segments, drawn by the user, allow the mean pixel intensity to be measured in particular regions throughout the image field. Typically, they are drawn over individual pixels or groups of pixels of a fiber optic sensor array, each of which contains a discrete sensor or portion of a gradient sensor. The script then enters a loop that steps through each frame, measuring the mean pixel intensity within each segment, and places the values in data columns. The

25   resulting columns can then be plotted to yield the temporal response of each sensor element of interest. Before plotting, however, responses are typically background-subtracted and then "standardized". In one embodiment, the initial background signal, prior to exposing the sensor to an analyte, is subtracted from all data points and then normalized by dividing each data point value by the maximum signal. In this embodiment, the maximum response has a value of 1.0. In an alternative embodiment, the data

30   points are "standardized" by dividing the data for each sensor response by the first point data point. In this embodiment, all responses are thus normalized to start at a value of 1.0 for the purpose of facilitating the graphical display of all the temporal responses. In an alternative procedure, the initial background signal, prior to exposing the sensor to an analyte, is adjusted to start at a value of 0.0 by subtracting the integer 1.0 from the standardized data points.

Conventional statistical analysis of sensor temporal responses may be employed where it is desirable to compare the discriminating capacities of either discrete copolymer sensors or gradient copolymer sensors for applications as individual sensors or as sensor elements in cross-reactive sensor arrays. Cluster analysis methods which employ graphical representations of statistically determined

5   dissimilarities between sensors are particularly useful in comparing sensor discriminating capabilities. Such statistical analytical methods are conventionally known and applied in the chemical arts and detailed discussion of these methods are provided by both R.G. Brereton [see *Chemometrics: Applications of Mathematics and Statistics to Laboratory Systems*, Ellis Horwood (New York, 1990), p. 244-266] and D. Livingstone [see *Data Analysis for Chemists*, Oxford Univ. Press (New York, 1995), p.

10   38-40, 81-92, 103-112, 170-174].

Cluster analysis methods may be employed for quantitative comparison of discrete sensor responses to specific analytes by characterizing the "dissimilarity" between the temporal responses of each copolymer sensor type to an analyte. While a variety of statistical measures are available for quantifying sensor dissimilarity, a particularly useful approach involves representing each sensor response as a

15   point in multidimensional space and then calculating the Euclidean distance between points [see T.A. Dickinson, et al., *Anal. Chem.* 69(17):3413(Sept. 1. 1997). In this method. the equation

$$ d_{ij} = \left[\sum_{1}^{T} (f_{i,t} - f_{j,t})^2 \right]^{1/2} $$

defines the statistical distance between the temporal responses of sensors i and j. The squared difference between the fluorescence signal from the two sensors at each time point, $f_{i,t}$ and f_t, is summed over all time points of the sensor response measurements. A diagonally symetrical distance

20   matrix is generated for the entire sensor array and the distance sare used to construct a dendrogram allowing the identification of groups or clusters having similar responses. A sample application of this method is provided in Example 6 for analysis of responses of discrete combinatorial copolymer sensors to benzene.

As an optional approach for quantifying dissimilarities of gradient sensor regions of interest to specific

25   analytes, a matrix of pairwise Euclidean distances may be generated as above for each ROI of a gradient sensor and a control and the distance values for each matrix are totaled to provide a comparison of an average summed distance for the gradient and control sensor regions of interest. A conventional multidimensional scaling plot may be utilized for graphical display of observed dissimilarity between the gradient and control sensors. This graphical method provides a two dimensional

30   representation of the pairwise dissimilarities between responses of regions of interest for each sensor type. A sample application of this method is provided in Example 7 for analysis of responses of gradient combinatorial copolymer sensors to benzene.

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In one preferred embodiment, the reduced sensor array data are employed in a neural network analysis for identifying analytes according to the method disclosed in White, et al., *Anal. Chem.* 68:2193-2202 (1996).

5. Sensor Element Signal Enhancement

5   In one alternative embodiment, to improve array detection response and sensitivity, the optical response signals from a large number of discrete sensor elements within each sensor type may be summed by simply adding the baseline-adjusted intensity values of all responses at each time point, generating a new temporal response comprised of the sum of all sensor responses for each sensor type. Signal summing can be performed in real time or during post-data acquisition data reduction and analysis. In

10   one embodiment, signal summing is performed with Excel (Microsoft, Redmond, WA), a commercial spreadsheet program, after optical response data are collected.

In a typical procedure, the standardized optical responses are adjusted to start at a value of 0.0 by subtracting the integer 1.0 from all normalized data points. Doing this allows the baseline-loop data to remain at zero even when summed together and the random response signal noise is canceled out.

15   The vapor pulse-loop temporal region, however, exhibits a characteristic change in response, either positive, negative or neutral, prior to the vapor pulse and often requires a baseline adjustment to overcome noise associated with drift in the first few data points due to charge buildup in the CCD camera. If no drift is present, typically the baseline from the first data point for each sensor element is subtracted from all the response data for the same sensor element. If drift is observed, the average

20   baseline from the first ten data points for each sensor element sensor is subtracted from all the response data for the same sensor element.

By applying this baseline adjustment, when multiple sensor element type responses are added together they can be amplified while the baseline remains at zero. Since all sensor elements respond at the same time to the vapor pulse, they all see the pulse at the exact same time and there is no registering

25   or adjusting needed for overlaying their responses. Cumulative response data are generated by simply adding all data points in successive time intervals. This final column, comprised of the sum of all data points at a particular time interval, may then be compared or plotted with the individual sensor responses to determine the extent of signal enhancement or improved signal-to-noise ratios.

EXAMPLE 1

30   A copolymer sensor array comprising discrete sensors formed by the combinatorial method of the present invention was made with two prepolymers, which were partially polymerized, and four discrete prepolymer composition ratios. An (80-85%) dimethyl-(1520%) (acryloxypropyl)methylsiloxane copolymer, PS802, available from Gelest Inc. (Tullytown, PA) and methyl methacrylate, MMA, available from Aldrich (Milwaukee, WI) were selected as example monomers for copolymerization. In one

35   experiment, a combinatorial sensor array was prepared from separate prepolymer solutions containing

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PS802/MMA combinations ranging from 0.0, 5.0, 20.0 and 50.0% MMA in PS802. In an alternative experiment, a second combinatorial sensor array was prepared from separate prepolymer solutions containing PS802/MMA combinations ranging from 0.0, 6.7, 33.3, and 50.0% MMA in PS802. In preparing each sensor array, dye solution of 1mg/mL Nile Red m chloroform and a 30mg/mL solution

5   of benzoin ethyl ether initiator in toluene was added to each prepolymer solution. For both arrays, the prepolymer mixture-dye-initiator solutions were individually polymerized onto the end of a coherent imaging fiber using the photodeposition system described herein, to form a plurality of discrete sensing regions across the face of the fiber. Each of the solutions was polymerized for 5 seconds, resulting in approximately 45µm-diameter polymer hemispheres or cones attached to the distal end of the fiber

10   bundle. Duplicates of each monomer combination mixture were polymerized, yielding a total of eight spatially separated sensor elements. A fluorescence image of the first sensor array comprising sensors containing 0.00, 5.0, 20.0 and 50.0% MMA in PS802 is shown in the PS802/MMA sensor array in Fig. 3. Replicate sensor elements were deposited for each of the prepolymer combination mixtures.

The second sensor array comprising sensors containing 0.00, 6.7, 33.3 and 50.0% MMA in PS802 was

15   tested by exposing the array to a variety of analytes. Figs. 4a-d depict the fluorescence output of this sensor array upon exposure to pulses of various saturated vapors. As shown in these figures, each of the four prepolymer combinations produces a unique temporal optical response for each of the four analytes tested: benzene (A), hexane (B), 2-propanol (C) and ethyl acetate (D). These results indicate that the optical responses for each copolymer sensor having a discrete prepolymer combination produce

20   a unique and distinguishable response to the same pulse of four different analytes and thus provide a characteristic discriminating measure of the presence of a variety of target analytes. Figs. 4a-d also indicate that the sensor response for each copolymer sensor type is reproducible and that replicates for each prepolymer combination produce similar response curves.

The diversity in copolymer sensor responses exhibited by the various copolymer sensor types shown in

25   Figs. 4a-d has utility for detecting and discriminating a variety of target analytes, demonstrating positive, negative, and biphasic fluorescence changes in response to each analyte tested. The individual optical response characteristics for each of the copolymer sensor types contains numerous additional distinguishing features which are useful for discriminating analytes as well, including different rise times, slopes, recovery rates, and sharp peaks associated with each sensor's characteristic response to each

30   of the analytes tested. As shown by the results of Figs. 4a-d, this discrimination capacity is seen both between classes of analytes, such as aromatic and alcohol compounds, as well as within a class of analytes, such as methanol and propanol.

From the results shown in Figs. 4a-d, it is important to note that there does not appear to be any obvious progression or linear relationship between the optical response characteristics of the prepolymer

35   combinations and the prepolymer composition ratios for each copolymer sensor type. The response characteristics of the combinatorial copolymer sensors are thus not simply related to the proportion of

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the two oligomers or monomers utilized. The differences observed in the optical response characteristics of these copolymer sensors is due to a difference in the polarity, swelling characteristics, or chemical absorption of the copolymerized matrices formed from each prepolymer combination. These results demonstrate that the combinatorial polymer sensors of the present invention provide

5   unique characteristic optical responses from each monomer combination, that such sensors exhibit a high degree of diversity in optical response to a variety of target analytes, that such sensors can discriminate between classes and within classes of target analyte compounds, that the sensor response is reproducible, and that the response characteristics are not simply related to proportional ratios of the oligomer or monomer combinations.

10   EXAMPLE 2

A copolymer gradient sensor was prepared by photodepositing a polymer strip from a prepolymer mixture having a time-varying oligomer or monomer composition. The distal end of a fiber optic bundle was immersed in 1 ml of a stirred solution containing PS901.5 (acryloxypropylmethyl siloxane), from United Chemical Technologies Inc. (Bristol, PA), in chloroform (1:1), with 27mg/mL of benzoin ethyl

15   ether initiator. For photodeposition of the copolymer, a UV light beam was focussed through a pinhole aperture and scanned across the face of the fiber optic bundle at a rate of 25µm/s while I ml of PS802 prepolymer solution (2:3 in chloroform, 30mg/mL BEE) was injected into the PS901.5 mixture using an Orion Research (Boston, Ma) Model M361 syringe pump. Both prepolymer solutions were flushed with nitrogen for 20 minutes prior to mixing and polymerization. The beam scanning and mixing of the two

20   prepolymer solutions occurred simultaneously and continuously over a twenty-two second photopolymerization period. This method of photodeposition produced a copolymerized polymer strip with a continuously varying structure and composition gradient due to the photodeposition of a prepolymer solution ranging from pure PS901.5 to a 5:4 mixture of PS901.5 and PS802. Using the same procedure, but without the addition of a second monomer to the reaction vessel, a

25   single-component polymer control strip of PS901.5 was polymerized onto the face of the same fiber optic bundle. Both of the photodeposited polymer strips were soaked in a Nile Red (Img/mL in toluene) solution for 30 minutes, rinsed with ethanol. and allowed to dry overnight.

For the resultant PS901.5/PS802 polymerized copolymer gradient sensor (Fig. 5a), twelve regions of interest (ROI's) were drawn at various locations along the vertical axes of both the copolymer gradient

30   sensor and an adjacent, single-component PS901.5 polymer control sensor stripe. The temporal fluorescence changes for each of the twelve ROI's of the copolymer sensor in response to vapor pulses of benzene (Fig. 6a) and methanol (Fig. 7a) were found to cover a wide range of shapes and intensities. The corresponding ROI's of the PS901.5 polymer control sensor (Fig. 5b), however, yielded twelve temporal responses of roughly the same shape in response to benzene (Fig. 6b) or methanol (Fig. 7b).

35   EXAMPLE 3

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A copolymer gradient sensor array was also prepared using a PS802/MMA monomer combination, according to the method described in Example 2. For this copolymer gradient sensor, a 1:1 solution of MMA monomer in chloroform was slowly added to a 1:1 prepolymer solution of PS802 in chloroform, each prepolymer solution containing 30 mg/ml of benzoin ethyl ether in toluene. During the continuous

5   and simultaneous addition and mixing of the MMA solution with the PS802 solution, a UV light beam was focussed through a pinhole aperture and scanned across the face of a fiber optic bundle which was immersed in the solution mixture, yielding a continuously varying, copolymer structure and composition gradient across the fiber bundle end face. This method of photodeposition produced a copolymerized polymer strip with a continuously varying structure and composition gradient due the photodeposition

10   of a prepolymer solution ranging from pure PS802 to a 1:1 mixture of PS802 and MMA.

For the resultant PS802/MMA polymerized copolymer gradient sensor (Fig. 8a), twelve regions of interest (ROI's) were drawn at various locations along the vertical axes of both the copolymer gradient sensor and an adjacent, single-component PS802 polymer sensor control strip. The temporal fluorescence changes for each of the twelve ROI's of the copolymer gradient sensor in response to

15   vapor pulses of hexane (Fig. 9a), methanol (Fig. 10a) and benzene (Fig. 11a) provide a broad diversity in optical response which is useful for discriminating a variety of target analytes. The corresponding ROI's of the PS802 polymer sensor control strip (Fig. 8b), however, yielded twelve temporal responses of roughly the same shape in response to the analytes hexane (Fig. 9b), methanol (Fig. I Ob), or benzene (Fig. 1 lb). Thus, direct comparison of the observed optical responses for the copolymer

20   gradient sensor and the polymer sensor control strip to the various analytes demonstrates the superior diversity in response and analyte discriminating capability of the copolymer gradient sensors and sensor arrays of the present invention.

EXAMPLE 4

The cluster analysis method was used to compare the dissimilarity of responses of the combinatorial

25   copolymer sensors of Example 1 to benzene. A dendrogram was constructed for identifying and isolating sensors having similar responses. The results are shown in the dendrogram of Fig. 12 where clusters of responses for similar copolymer sensor composition are shown for 0% MMA (1 and 2), 6.7% MMA (3 and 4), 33.3% MMA (5 and 6) and 50% MMA (7 and 8) and dissimilarity of responses between cluster groups are shown for the different copolymer sensor compositions. Fig. 12 also demonstrates

30   the non-linear relationship between copolymer composition and sensor response where the responses of sensors with a 50% MMA composition are more closely related to the responses of sensors with 0% and 6.7% MMA compositions than the 33.3% MMA composition.

EXAMPLE 5

The cluster analysis method was used to compare the dissimilarity of responses of the combinatorial

35   copolymer gradient sensor and control sensor of Example 2 to benzene. The responses of twelve regions of interest for each sensor type were compared in a multidimensional scaling plot for

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representing the increased dissimilarity between the gradient sensor and control sensor regions of interest. The results are shown in the multi-dimensional scaling plot of Fig. 13 where pairwise dissimilarities between regions of interest for each sensor type are plotted. As demonstrated by this plot, the gradient sensor responses are more widely scattered than the control sensor, indicating greater

5   diversity in sensor response.

Having described the preferred embodiments of the invention, it will now become apparent to one of skill in the art that other embodiments incorporating the concepts may be used. Therefore, it is not intended to limit the invention to the disclosed embodiments but rather the invention should be limited only by the spirit and scope of the following claims.

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What is claimed is:

1. A sensor array comprising:

   a) a substrate comprising discrete sites; and

   b) a plurality of copolymer sensors distributed on said sites, each of said sensors comprising

5 a discrete mixture of at least a first and a second polymerizable material.

2. A sensor array according to claim 1 wherein said copolymer sensors are on microspheres distributed on said sites.

3. A sensor array according to claim 1 or 2 wherein said substrate is a fiber optic array.

4. A sensor array according to claim 1, 2 or 3 wherein said sensors further comprise at least one dye.

10 5. A sensor array according to claim 1, 2, 3 or 4 further comprising a detector for detecting sensor responses.

6. A sensor array according to claim 5 wherein said detector is an optical detector.

7. A method of detecting the presence of a target analyte in a sample comprising:

   a) adding said sample to a sensor array comprising:

15 i) a substrate comprising discrete sites; and

   ii) a plurality of copolymer sensors distributed on said sites, each of said sensors comprising a discrete mixture of at least a first and a second polyermizable material;

   b) detecting an alteration in a property selected from a group consisting of mass, temperature, heat, light, voltage, current, polarity, intensity, refractive index, polarization, phase, wavelength,

20 frequency, periodicity, and dimension.

8. A method according to claim 7 wherein said said copolymer sensors are on microspheres distributed on said sites.

9. A method according to claim 7 or 8 wherein said substrate is a fiber optic array.

10. A method according to claim 7, 8 or 9 wherein said sensors further comprise at least one dye.

25 11. A method according to claim 7, 8, 9 or 10 further comprising a detector for detecting sensor responses.

12. A method according to claim 11 wherein said detector is an optical detector.

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13. A method of making a sensor array comprising:

    a) polymerizing in a plurality of predetermined ratios:

        i) a first solution comprising a first polymerizable material; and

        ii) a second solution comprising a second polymerizable material;

5   said ratios being unique for at least two of said solutions, such that each polymerization reaction results in a different copolymer sensor; and

    b) distributing said copolymer sensors on a substrate.

14. A method according to claim 13 wherein said substrate comprises a fiber optic array.

15. A method according to claim 13 wherein said copolymer sensors are polymerized onto 10 microspheres that are distributed on said substrate.

16. A method according to claim 13 wherein said copolymer sensors are polymerized into microspheres that are distributed on said substrate.
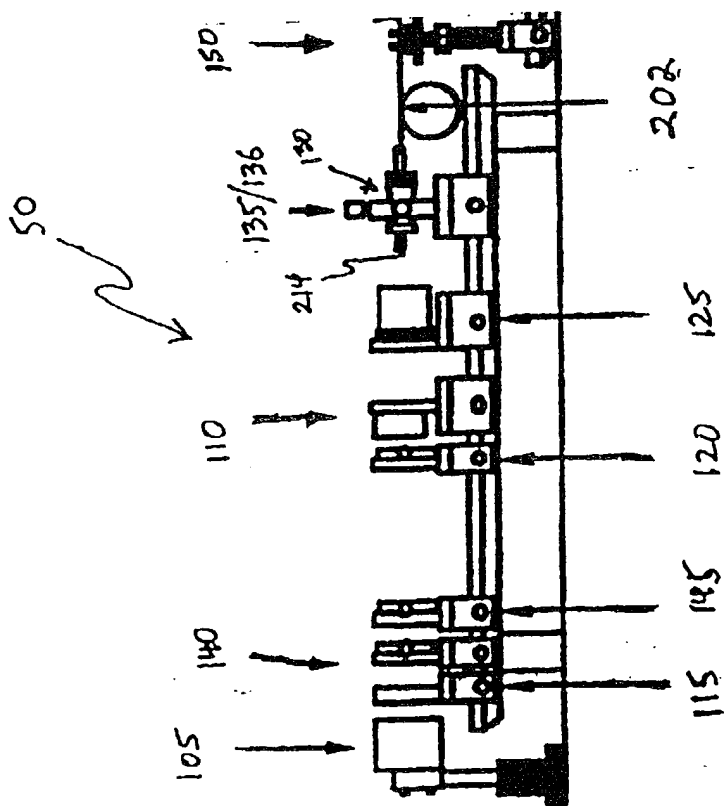
Fig. 1
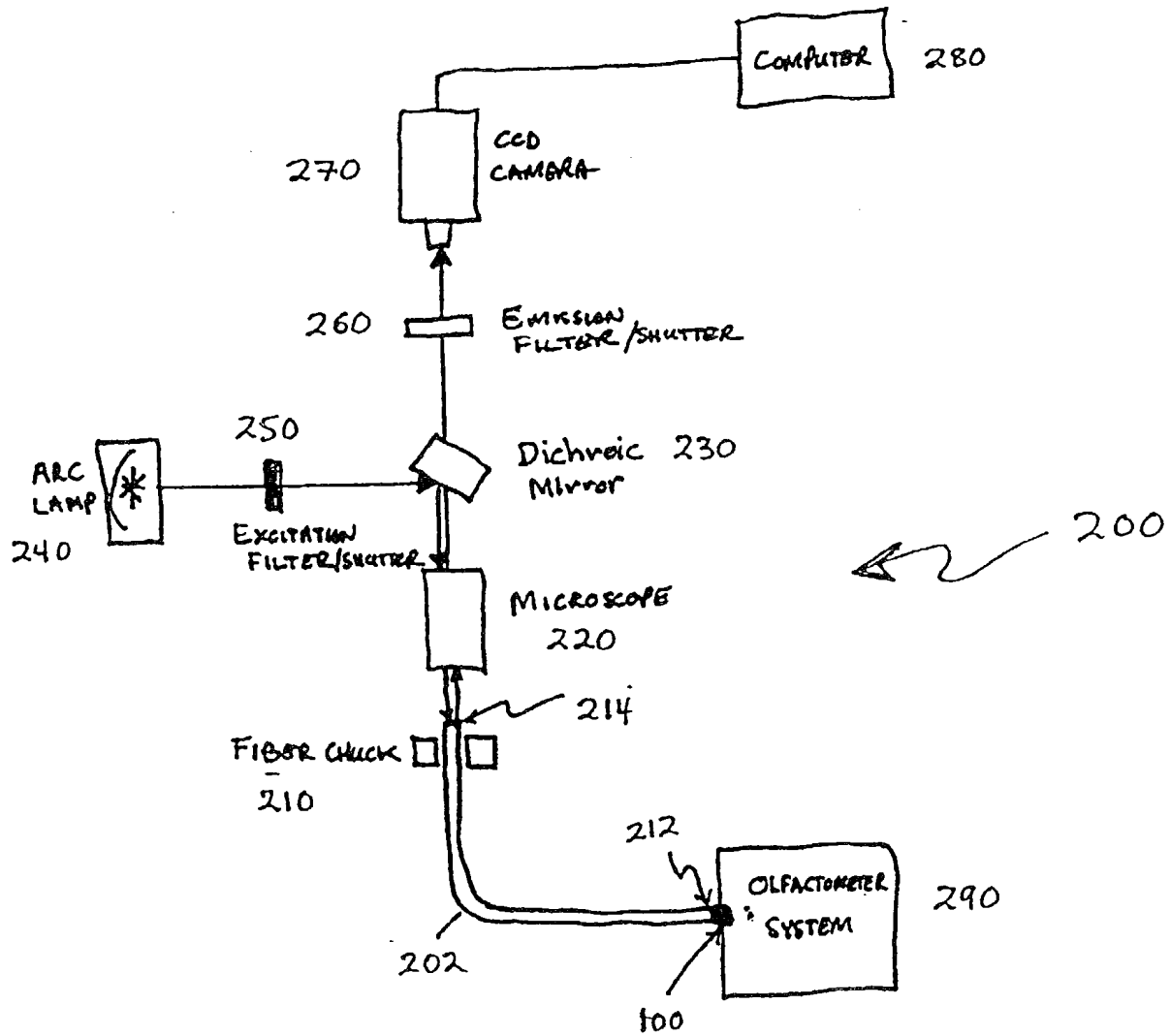
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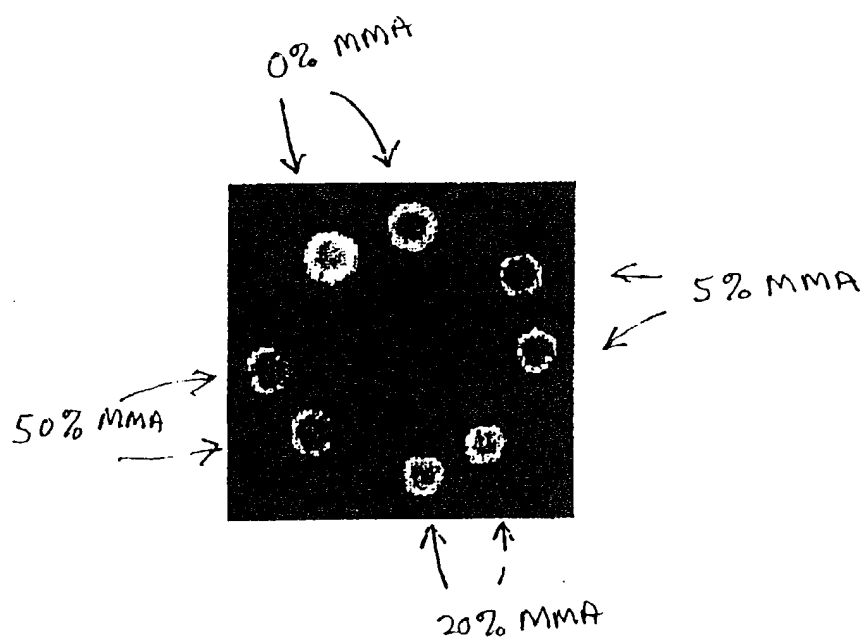
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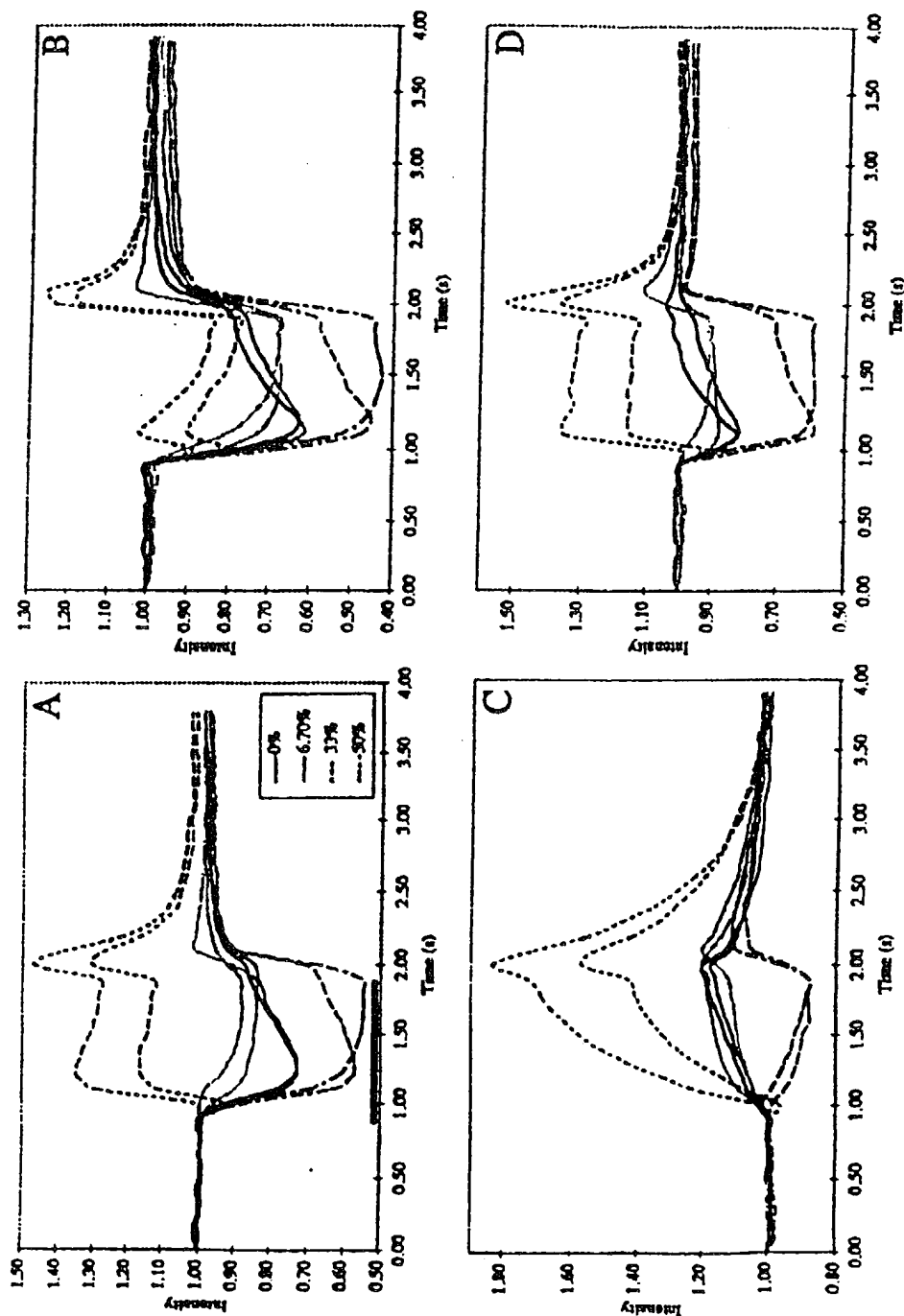
Fig. 4a-d
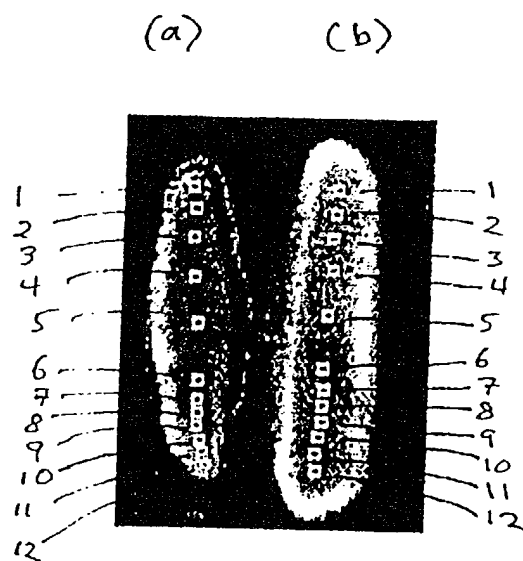
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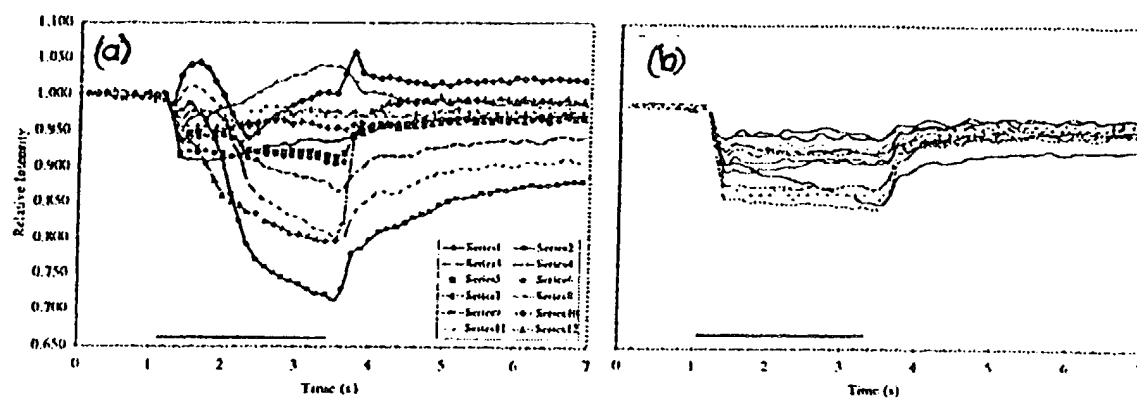
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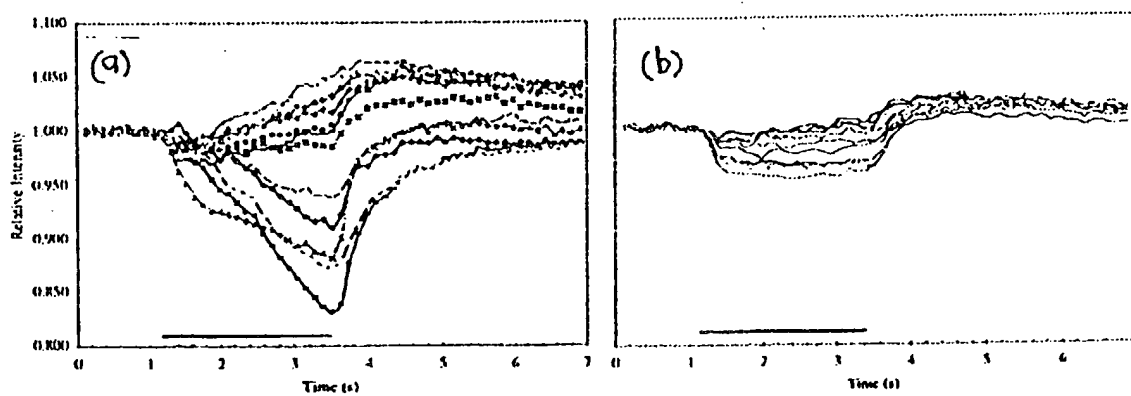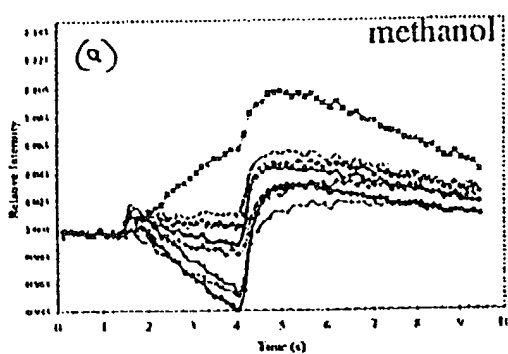

Fig. 7a-b

(a)   (b)
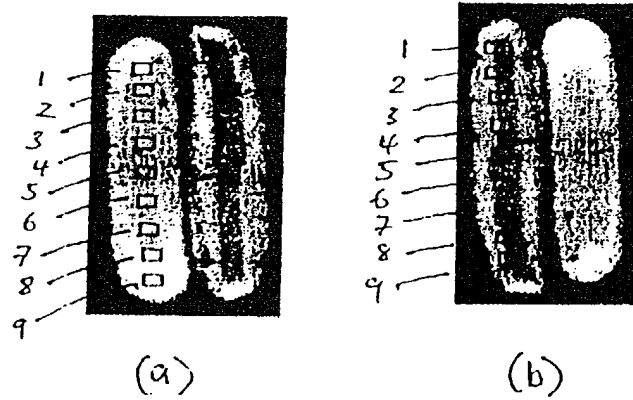
Fig. 8a-b
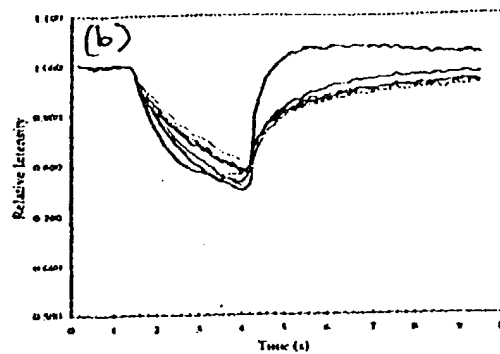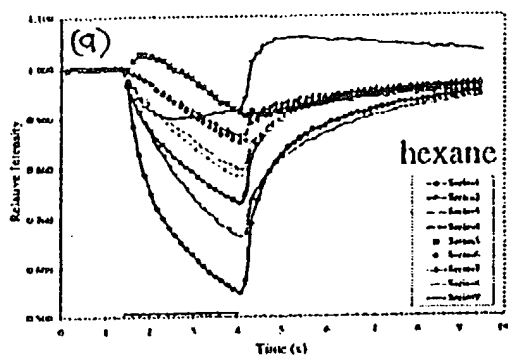
Fig. 9a-b
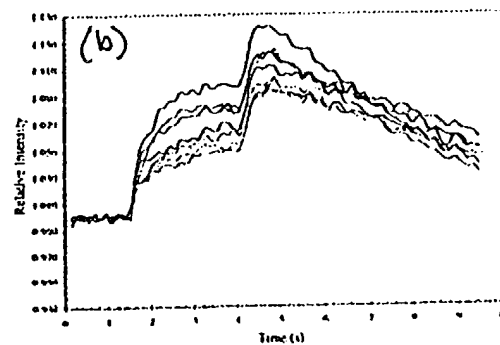
Fig. 10a-b

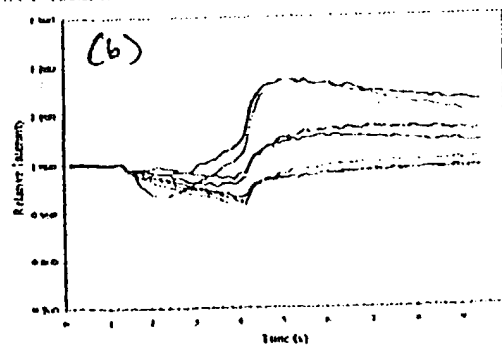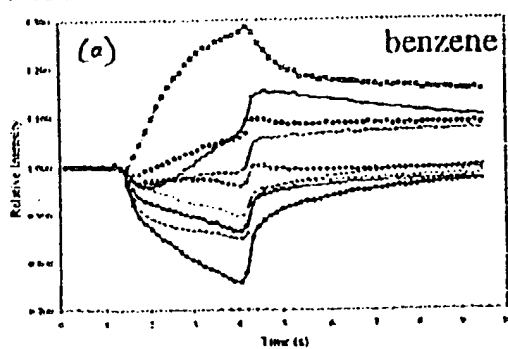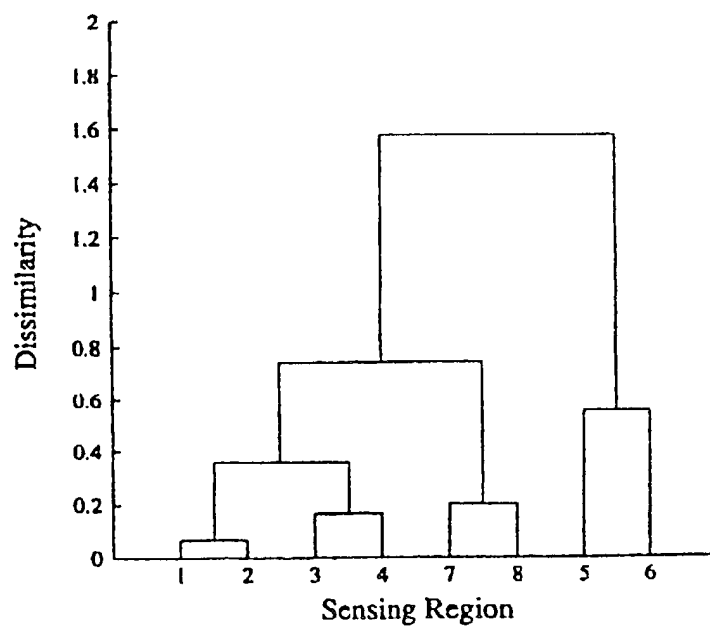

Fig. 11a-b

Fig.12
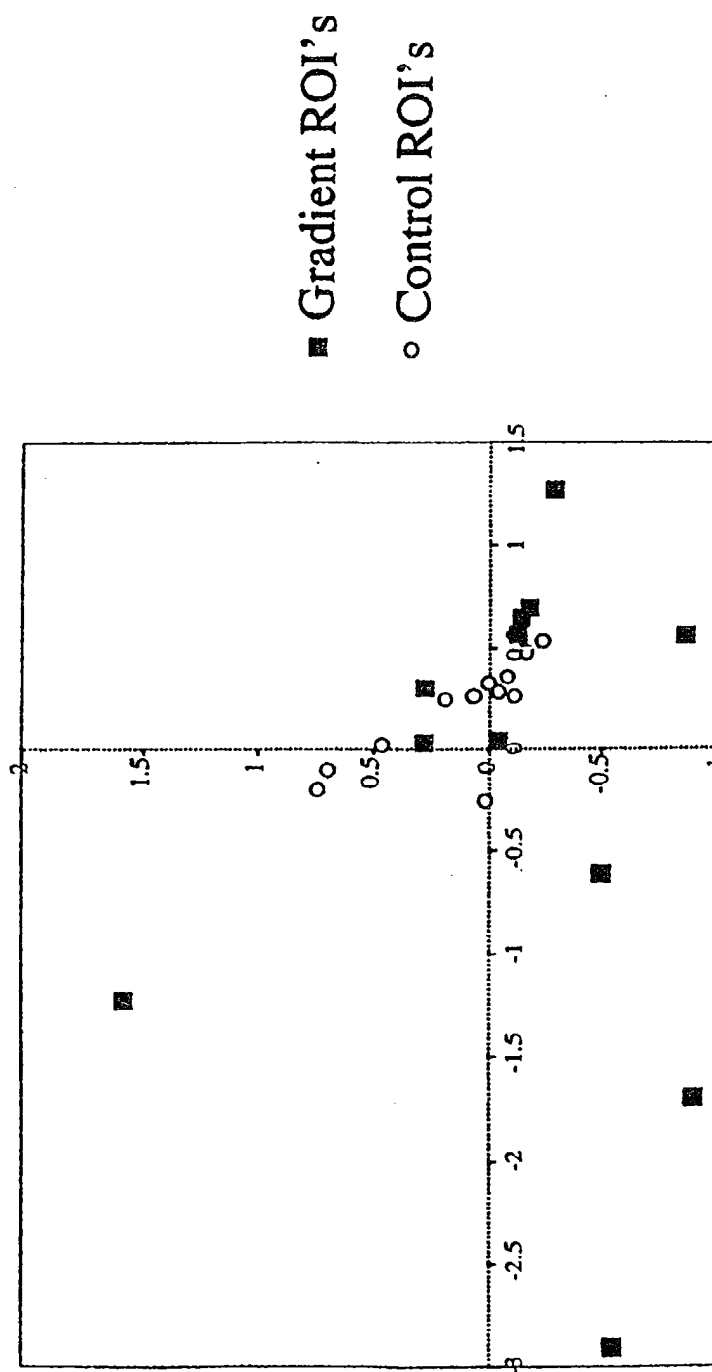
Multi-Dimensional Scaling Plot
Gradient vs. Control Stripe

■ Gradient ROI's

○ Control ROI's

Fig. 13